UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4799

APPROVAL SHEET

Title of Dissertation: "The Effects of Low Density Lipoproteins on Endothelial Mediated Vasoactivity in the Coronary Circulation in Swine"

Name of Candidate: Mark Crago
Doctor of Physiology Degree
27 March 1998

Dissertation and Abstract Approved:

James Terris, Ph.D.
Department of Physiology
Committee Chairperson

3/27/98
Date

Jack McKenzie, Ph.D.
Department of Physiology
Committee Member

3/27/98
Date

Terez Shea-Donohue, Ph.D.
Department of Medicine
Committee Member

3/27/98
Date

Chu-Shek Lo, Ph.D.
Department of Physiology
Committee Member

3/27/98
Date

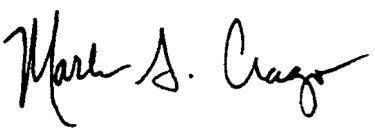
Donald Seilitti, Ph.D.
Department of Medicine
Committee Member

Date

The author hereby certifies that the use of any copyrighted material in this thesis manuscript entitled:

"The Effects of Low Density Lipoproteins on Endothelial Mediated Vasoactivity in the Coronary Circulation in Swine"

beyond brief excerpts is with the permission of the copyright owner, and will save and hold harmless the Uniformed Services University of the Health Sciences from any damage which may arise from such copyright violations.



Mark S. Crago
Department of Physiology
Uniformed Services University of the Health Sciences

ABSTRACT

Title of Dissertation: The Effects of Low Density Lipoproteins on Endothelial Mediated Vasoactivity in the Coronary Circulation in Swine

Mark S. Crago, Doctor of Philosophy, 1998

Dissertation Directed by: Dr. Jack E. McKenzie, Ph.D., Professor
Department of Physiology

Hypercholesterolemia, which includes high concentrations of low density lipoproteins (LDL), alters normal endothelial function in patients with atherosclerosis. The aim of this study was to investigate the effects of low density lipoproteins on endothelial mediated changes in coronary blood flow and coronary vascular resistance in hypercholesterolemic swine. Animals were assigned at random into three groups (n=5/group). A control group received normolipic diet and a hyperlipidemic group received a lipid-enhanced diet. The third group received the lipid-enhanced diet as well a surgical procedure to induce mechanical damage in the left anterior descending coronary artery. Significance was set at the $p \leq 0.05$ level. LDL cholesterol was significantly higher in the high cholesterol (116±23 mg/dl) and high cholesterol plus denudation (283±57 mg/dl) groups than in control (48±1 mg/dl). Both mean arterial and diastolic blood pressures were significantly higher in the hyperlipidemic group (110±5.1 and 101±4.8 mmHg respectively) as compared to control (88±3.6 and 81±3.6 mmHg respectively). A significant linear relationship was found between the LDL concentration and diastolic blood pressure. Acetylcholine, substance P, adenosine, and nitroglycerin were injected directly into the left anterior coronary artery and changes in coronary blood flow were measured using a perivascular Doppler flow probe. Global resistance was calculated as the pressure difference between the aortic root pressure and coronary sinus pressure divided by coronary blood flow. Focal resistance was calculated as the pressure difference between the aortic root pressure and mid-left anterior descending coronary artery pressure divided by coronary blood flow. Baseline coronary blood flow and resistance to coronary blood flow prior to drug injection showed no significant differences between groups.

Intracoronary injections of nitroglycerin, adenosine, and substance P showed a significant increase in coronary blood flow from baseline in all groups. These increases in coronary blood flow correlated with a reduction in global resistance to coronary blood flow from baseline. Changes in coronary blood flow and global coronary resistance in response to these drugs between the control and hyperlipidemic groups were not significant. Significant differences in global resistance to coronary blood flow from baseline between the control and hyperlipidemic groups were seen with acetylcholine 0.01 μg/ml (+1.5±0.7 mmHg·ml^{-1}·min. vs. -1.4±0.4 mmHg·ml^{-1}·min., hyperlipidemic vs. control respectively). Additionally, acetylcholine 1.0 and 2.0 μg/ml produced a significantly greater hyperemic response over baseline following coronary vasoconstriction in the hyperlipidemic group (+35.8±11.9 ml/min/100g) compared to control (+9.0±1.8 ml/min/100g). Using the hyperlipidemia plus denudation group, similar changes in focal resistance were noted in the hyperlipidemia and hyperlipidemia plus denudation groups when compared to control in response to acetylcholine and substance P injections. Both the hyperlipidemic and hyperlipidemic plus denudation groups showed increases from baseline in focal resistance with substance P 1.0 μg/ml (+0.081±0.05 and +0.067±0.065 mmHg·ml^{-1}·min. respectively), acetylcholine 0.01 μg/ml (+0.16±0.15 and +0.018±0.004 mmHg·ml^{-1}·min. respectively) and acetylcholine 0.10 μg/ml (+0.018±0.009 and +0.003±0.007 mmHg·ml^{-1} ·min. respectively). The control group showed decreases in focal resistance in response to these acetylcholine and substance P doses. These results support the hypothesis that hyperlipidemia may be related to impaired endothelium dependent vasodilation in swine.

The Effects of Low Density Lipoproteins on Endothelial Mediated Vasoactivity in the Coronary Circulation in Swine

by

Mark S. Crago

Dissertation submitted to the faculty of the Department of Physiology
Graduate Program of the Uniformed Services University of the Health Sciences
In partial fulfillment of the requirements for the degree of
Doctor of Philosophy, 1998

DEDICATION

This dissertation is dedicated to the following individuals to whom I am eternally grateful for their love and support:

This work, as is the rest of my life, is dedicated to my wife, Karen Ziemba Crago. If it were not for her constant love, support and dedication to me and our family, I do not know how it all could have been completed.

I also dedicate this work to my daughter, Morgan Ashley Crago. She has often showed me what is truly important in this life.

I would also like to dedicate this project to my family, specifically my mother, Joan Roberts Crago and my late father, William Hoffman Crago. They have supported me in all that I have ever done, since day one.

ACKNOWLEDGMENTS

To Dr. Jack E. McKenzie, my mentor, and even more importantly, my friend. His energy and enthusiasm was not only appreciated, but was needed in order for me to complete this project. His guidance and experience allowed me to do things I never thought possible. I will never think of USUHS without thinking of him and the good times we had in this lab. I owe him a debt of gratitude that I probably will never be able to repay. You have been an example to me on how I should balance time spent in the lab as well as time with my family.

I would also like to recognize the efforts of Shanda West in making my dissertation a reality. Thanks for putting up with all of my "requests" and for doing all of the little things that need to be done in order to get a project completed. I would also like to thank Karen Hoeprich for her assistance in organizing more information and data than she ever had in mind when coming to our lab as an intern.

I am also grateful for the assistance and guidance provided to me by Drs. Terez Shea-Donohue, Tony Lo, Jim Terris, and Don Sellitti. You have helped me to put together a dissertation in a short amount of time - not an easy task, and one I thank you for doing for me.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ACH	Acetylcholine
ADO	Adenosine
ANOVA	Analysis of variance
atm	Atmosphere
ATP	Adenosine triphosphate
CAD	Coronary artery disease
CBF	Coronary blood flow
cGMP	Cyclic guanosine monophosphate
CTL	Control group
CO_2	Carbon dioxide
dP/dT	First derivative of pressure (change in pressure over time)
DRC	Dose response curve
ECG	Electrocardiogram
EDRF	Endothelium derived relaxing factor
F	French
G	Gauge
GTP	Guanosine triphosphate
HC	Hypercholesterolemic group
HC+D	Hypercholesterolemic plus denudation group
HCT	Hematocrit
HDL	High density lipoprotein
HR	Heart rate
IDL	Intermediate density lipoprotein
IM	Intramuscular
IV	Intravenous
LAD	Left anterior descending coronary artery
LDL	Low density lipoprotein
MABP	Mean arterial blood pressure
mmHg	Millimeters of mercury
µg/ml	Micrograms per milliliter
NO	Nitric oxide
NTG	Nitroglycerin
O_2	Oxygen
oxLDL	Oxidized low density lipoprotein
PRP	Pressure rate product
SEM	Standard error of the mean
SP	Substance P
VLDL	Very low density lipoprotein
VSM	Vascular smooth muscle

SIGNIFICANCE

Atherosclerosis is a form of cardiovascular disease that is responsible for almost half a million deaths in the United States, making it the number one cause of death in Western society [1,2]. While the mortality associated with atherosclerosis, or coronary artery disease (CAD), is very high, the morbidity related to the disease is staggering. The cost of treating patients with CAD is measured in the billions of dollars [3].

Atherosclerosis is a condition in which fibrous or cholesterol-laden plaques form along the walls of the more proximal portions of the major coronary arteries. These plaques usually develop over many years, not uncommonly beginning as early as the adolescent years in the form of "fatty streaks" along the coronary endothelium [4]. These plaques develop over time, stimulated by a variety of conditions, and eventually disrupt the normal flow of blood through the diseased artery. This disruption of flow leads to an imbalance in the delivery of oxygen to the working myocardium in relationship to the demand for oxygen [5]. The myocytes require a large amount of molecular oxygen in order to function normally. This is best illustrated by the fact that 25-40% of the volume of the cell is dedicated to the production of adenosine triphosphate (ATP), the major energy-storing molecule produced by the mitochondria of the myocyte [6,7]. The imbalance between oxygen supply and demand, clinically referred to as ischemia, is initially manifest in the individual with CAD in the form of angina pectoris, or what is commonly referred to as cardiac chest pains. The development of angina is often one of the most clinically important symptoms and indicates the presence of one or more flow-limiting plaques in the coronary arterial lumen. During ischemia,

there is a sharp decline in the production of ATP since aerobic glycolysis is dependent upon the presence of molecular oxygen [8]. Small amounts of ATP can be created anaerobically, but not in sufficient quantities for the myocytes to remain viable. Concentrations of creatine phosphate, a secondary source of energy in the myocyte, are also quickly depleted in ischemia. In two minutes of ischemia, levels of creatine phosphate are depleted by up to 80% [9,10]. With such declines in both forms of high energy phosphates during ischemia, systolic function of the ventricles, especially of the left ventricle, rapidly declines. Within a few seconds of the cessation of myocardial blood flow, both systolic and diastolic functions of the heart are compromised, as seen in declining development of pressure in the ventricles as well as falling compliant pressure volume curves [5]. In the ischemic zone, the central area of the myocardium directly affected by the loss of coronary blood flow, loss of all contractile function is observed within 10-15 seconds after the onset of ischemia [11]. Only if the cause of ischemia is corrected and a return to normal coronary blood flow occurs will normal ventricular function be returned. If the ischemia persists and no clinical intervention occurs, myocardial infarction with ensuing loss of all cardiac function develops, leading to the death of the individual.

Mechanisms of Atherosclerotic Inhibition of Coronary Blood Flow

The mechanisms by which the atherosclerotic plaque can disrupt coronary blood flow are varied, but can be grouped into three different categories: (1) rupture and thrombotic occlusion, (2) physical obstruction, and (3) coronary vasospasm. Rupture of the atheroma and occlusion of a major extramural

coronary artery by thrombotic occlusion has been observed and documented since the early twentieth century [12]. The cause of the rupture of the plaque and its relationship to the plaque components is now becoming clearer with the recent availability of information regarding plaque development [13]. However, it is still debated whether the coronary artery thrombi are the cause of, or are the result of, acute myocardial infarction [14].

Another mechanism by which the plaques can occlude coronary blood flow is by growing to such a large size as to physically occlude blood flow. Although these plaques are not usually responsible for the development of angina, a number of recent studies have shown these plaques to be the cause of acute myocardial infarction [15, 16, 17]. One of the more notable results of these studies is that large plaques causing greater than 70% stenosis are the least common means for occluding coronary blood flow. These investigators found that the most common link to myocardial infarction was the development of a coronary stenosis that occluded the artery by less than 50%. Indeed, Falk and coworkers showed that 65% of the patients who developed a myocardial infarction had lesions that occluded the artery by 50% or less. [18]

The recent accumulation of research showing relatively small plaques being responsible for the majority of myocardial ischemic events has led to an understanding of the third means by which coronary blood flow can be inhibited. The sudden development of coronary vasospasm, where severe vasoconstriction is seen in large epicardial vessels, can reduce normal coronary blood flow and can be demonstrated angiographically.

The vascular endothelium of the tunica intima of the coronary arteries plays a dynamic part in allowing the vessel to properly vasodilate or

vasoconstrict. [19, 20] Thus, any alterations of the coronary arteries that would interfere or disrupt normal endothelial structure would then be a candidate for changing normal coronary vasomotor ability.

Possible Mechanisms of Atherogenesis

While the precise mechanism of atherogenesis is not known, nor is it likely monofactoral, several hypotheses have been introduced to explain the origins of plaque development. In 1973, Ross and Glomset formally proposed the theory that stated in order for atherogenesis to begin, some type of initial endothelial damage must occur [21]. This "Response to Injury" theory has been modified since its inception, but the premise that the initial trigger for plaque development is endothelial damage remains [4]. Increasing age, smoking, diabetes, high blood pressure, genetics, and high cholesterol diets are strongly related to atherosclerosis [22].

While all of the previously mentioned conditions are implicated as possible mechanisms which might trigger the initial endothelial damage precipitating atherogenesis, the low density lipoprotein (LDL) molecule is often at the top of the list. Since the early 1970's, many investigators have not only suggested that the LDL particle fosters plaque development but also that LDL is a probable candidate for initiating atherogenesis [23, 24, 25]. The LDL particle is responsible for cholesterol transport to all of the cells of the body. Cholesterol plays a significant role in the structure of cell membrane, including both the plasma membrane and the membrane-bundled cell organelles. Steroid synthesis is also dependent upon

cholesterol, since all steroid hormones are derived from enzymatic modification of the cholesterol molecule.

The LDL particle is one of five major families of lipoproteins that are found in the plasma. It is a byproduct of the hydrolysis of very low density lipoproteins (VLDL), one of the other four lipoproteins found in the serum. Human LDL is strictly defined as the population of lipoproteins that can be isolated via ultracentrifugation with a density range of 1.019 to 1.063 g/ml [26]. While the other byproduct of VLDL hydrolysis, intermediate density lipoprotein (IDL), has a half life of only a few hours, the LDL particle remains in the circulation for about two days before it is cleared from the circulation [26]. Normolipidemic persons usually have serum LDL levels of approximately 3 mg/ml, carrying approximately 60 % of the total serum cholesterol [27]. The LDL particle contains only one major form of protein, the apolipoprotein B100 (B100). The core of the LDL particle contains predominantly cholesterol esters, but insignificant concentrations of triglycerides are also present. Surrounding the core of esterified cholesterol is a monolayer consisting of amphipathic phospholipid molecules (mostly phosphatidylcholine), apolipoprotein B100, and small amounts of free cholesterol.

Cholesterol transport is a receptor-mediated process that requires B100 interacting with cell-surface LDL receptors [28]. Cholesterol transport from the LDL particle to cells expressing the LDL receptor (the highest concentration of these cells is seen in the liver) is dependent upon both normal LDL receptor function and B100 structure. Genetic alterations of the B100 gene as well as mutations of the LDL receptor gene have been demonstrated and positively linked to the development of coronary artery disease [29, 30].

The LDL particle travels throughout the systemic circulation to provide cholesterol to all of the cells of the body through a receptor-mediated process, as previously mentioned. However, as the LDL moves through blood vessels, in particular, the arteries, it can become entrapped within the vessel wall. Specifically, the LDL particle is trapped within subendothelial spaces between the tunica intima and the tunica media [31]. Kruth *et al* were the first to reveal that LDL particles can become fixed inside intact arterial walls [32]. This was explicitly demonstrated by Frank and Fogelman who, by using ultrastructural techniques, showed the LDL particle embedded in the mesh-like fibers in arterial wall cells [31]. The transport of the LDL particle into the subendothelial space has been demonstrated to be rapid, resulting in higher concentrations of B100 inside the arterial wall than are found in the serum [33, 34]. Certain sites along arterial vessels have been found to be more susceptible to LDL penetration and accumulation, thus being deemed as sites predisposed to develop atherosclerosis [35, 36]. Demer and coworkers have shown that mechanical and hemodynamic factors may play a role in a vessel's predisposition to atherogenesis [36]. Early atherogenesis at these sites of predisposition has been documented in both animal models as well as human subjects [37-39].

LDL accumulation in arterial wall cells sets the stage for the next process in atherogenesis which may be the cause of, as well as the fuel for, plaque formation and growth [26]. Low density lipoprotein oxidation changes the structure of the particle, making it incapable of participating in the normal receptor-mediated process of cholesterol transport [40]. Fogelman *et al* showed that there is an accumulation of oxidatively modified LDL particles (oxLDL) in atherosclerotic plaques [41]. These researchers not only identified oxLDL as a

constituent of the plaque, but were also able to demonstrate that oxLDL led to cholesterol accumulation in monocyte/macrophages. This finding was important in that it had been shown about a decade earlier that atherosclerotic plaques contained "foam cells", or lipid-laden macrophages [42, 43]. These foam cells form when circulating monocytes enter the arterial walls and phagocytize the oxidatively modified LDL particle in a receptor-independent pathway allowing for a non-regulated accumulation of cholesterol in the macrophages, further decreasing lumen diameter [40]. Chemoattractant molecules and cytokines released by the altered endothelium, accumulated macrophages, or possibly even vascular smooth muscle cells, attract additional circulating monocytes and further accelerate foam cell production [44]. OxLDL can also cause endothelial damage directly, in that oxLDL is cytotoxic to the surrounding cells in the arterial wall, including the vascular endothelium [4].

The initial damage to the endothelium can be subtle, eliciting a series of cellular reactions that culminate in the development of a fatty streak, the precursor of atherosclerotic plaques. This initial, minor alteration of the endothelium has recently been suggested to occur in areas of *intact* endothelium. Thus the accumulation of lipid in the subendothelial spaces is a likely cause of the initial changes in otherwise normal vascular endothelium [39, 45].

Endothelium-Mediated Coronary Vascular Tone

Of the numerous functions of the vascular endothelium, its role in maintaining normal vascular tone by the production and release of small vasoactive molecules such as nitric oxide (NO) and prostacyclin is critical in the

coronary circulation. Investigations into nitric oxide and its role in maintaining normal coronary vascular tone have been intense ever since it was found to be a significant factor in vasoactivity [20]. Known also as endothelium dependent relaxing factor, or EDRF, NO is an uncharged biologic messenger that readily traverses the endothelium and reaches the vascular smooth muscle (VSM). It was the work of Palmer and coworkers that led to the conclusion that EDRF was actually nitric oxide [46]. In the arteries, NO plays a major role in vasodilation, inhibition of platelet activation, inhibition of leukocyte adhesion to the endothelium, and cessation of smooth muscle cell proliferation [47]. Nitric oxide is produced by the vascular endothelium covering the tunica media, triggered by various pharmacologic and mechanical stimuli, and diffuses into the VSM. In the VSM, NO activates the conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP) by soluble guanylate cyclase. As the concentrations of cGMP increase in the VSM, the concentrations of intracellular calcium decline due to the activation of membrane-associated calcium/magnesium ATP pumps [48]. This decline in intracellular calcium in the VSM permits smooth muscle relaxation, and thus vasodilation.

There are numerous stimuli that will elicit the production of NO from the vascular endothelium. Chemical means include acetylcholine, serotonin, substance P, histamine, adenosine triphosphate (ATP), bradykinin, and thrombin. Shear stress and hypoxia are non-pharmacologic means which have also been shown to cause NO production [47]. In the coronary vascular bed, NO has been shown to play a major role in the control of coronary blood flow [49].

Metabolic Regulation of Coronary Blood Flow

Coronary blood flow is principally regulated by the demand of the myocardium for metabolic oxygen to fuel its intense demand for energy in the form of high energy phosphates. As cardiac work increases, the demand for oxygen increases, and as a result, the flow of blood to the cardiac muscle increases. The mechanism behind this increase in coronary blood flow is linked to the production of a variety of metabolic metabolites that facilitate vasodilation. Ardehali and Ports list numerous metabolic vasodilators of physiologic importance, including the hydrogen ion, potassium ion, adenosine, lactate, histamine, oxygen, carbon dioxide, and several polypeptides [50]. Adenosine is a particularly potent vasodilator *in vivo* [51, 52]. McKenzie *et al* showed that adenosine is responsible for the increase in coronary vasodilation as the work of the heart increased [53]. Under conditions where there is a decrease in coronary blood flow, or when there is an increase in the resistance to coronary blood flow, hypoxic conditions can result if the supply of oxygen is insufficient to meet the demand. Adenosine accumulation during the ischemic event allows for the resultant "hyperemic response" to the ischemic period by inducing a significant increase in blood flow (or a decrease in the resistance to coronary blood flow) after the cause of the ischemia is removed [54]. Thus the hearts ability to modulate the resistance to blood flow is critical in meeting the metabolic demands of the myocardium.

Adenosine has an estimated half-life of less than 10 seconds in the circulation. Its mechanism of action includes enhanced potassium conductance and inhibition of cyclic adenosine monophosphate (cAMP) induced calcium

influx. Intracoronary adenosine facilitates smooth muscle hyperpolarization and suppression of the calcium dependent action potential which drives smooth muscle contraction, leading to vasodilation. Berne proposed that in the coronary circulation adenosine acts as an intercellular messenger between the cardiac cell and the vascular smooth muscle [55]. Since adenosine is a metabolite of ATP, as the heart works harder, adenosine accumulates in the interstitial space adjacent to the VSM as the high energy phosphates are cleaved, leading to increases in coronary blood flow.

Compounds That Elicit NO Production

In this study, two endothelium-dependent vasodilators were chosen for their abilities to induce NO production through an endothelial receptor-dependent mechanism. Acetylcholine (ACH) has been used in both animal and human studies to investigate endothelial function [56-59]. When administering ACH, a concentration dependent effect can be demonstrated in the coronary arteries of a variety of species including the pig [60, 61], dog [62] and humans [58]. At low concentrations (0.01μg/ml), ACH acts through muscarinic receptors in endothelial cells to produce NO production, thus allowing for a decrease in the resistance to coronary blood flow [63]. As the concentration is increased to 1.0 μg/ml, ACH has been shown to act directly on vascular smooth muscle to produce vascular smooth muscle contraction [56, 57, 64]. Substance P (SP), a tachykinin, also elicits the production of NO by the endothelium resulting in VSM relaxation through an endothelium-dependent mechanism [65, 66]. Removal of the endothelium in canine coronaries was shown to remove the vasodilatory effects of SP administration [69].

It has been demonstrated that both ACH and SP are effective in triggering intact vascular endothelium to produce NO in the coronary circulation of the swine [67, 68]. If the endothelium is damaged or altered, studies have shown constricting effects with doses of ACH that in normal coronary arteries produced dilation [56, 57]. Cohen and coworkers showed that porcine coronary artery segments taken from hypercholesterolemic swine demonstrated VSM contraction with doses of ACH that caused VSM dilation in the control [56]. Other studies using non-human primates demonstrated the same phenomenon [64].

Hypercholesterolemia and its Effects on Blood Flow

The condition of hypercholesterolemia is one of four classes of disease referred to as dislipidemias. In hypercholesterolemia, serum levels of total cholesterol, specifically LDL, are significantly elevated above normal (normal LDL concentrations being ≤ 125 mg/dl in humans, and ≤40 mg/dl in swine). Both oxLDL and native LDL have been implicated in damaging the endothelium in such a way so as to alter normal endothelial function [70, 71]. A report by Andrews *et al* showed that non-oxidized LDL in physiologic concentrations had the ability to inhibit normal endothelial dependent relaxation [71]. Anderson and coworkers also showed that there was a relationship between coronary vasomotion and the susceptibility of LDL oxidation [58]. Additional studies have shown that cholesterol-reducing medications have been effective in returning normal endothelial mediated function in both animal and human studies [72, 73, 74].

The time course for these changes in endothelial function to take place has become an area of intense research. It has been well documented that

atherosclerosis is related to serum cholesterol and LDL concentrations [75, 76], but atherosclerosis takes years to develop. Many are now becoming interested in trying to determine the short-term effects of high-fat meals on endothelium dependent vasomotion. In a recent report, Plotnick *et al* were able to show that a single high-fat meal was able to temporarily inhibit normal endothelial-dependent relaxation in brachial arteries of human subjects [77]. Following a high fat meal, flow mediated vasodilation fell significantly at 2, 3, and 4 hours compared to baseline flow. They also showed improvement in vascular function in patients receiving an antioxidant prior to the high fat meal (compared to the patients who ate the fatty meal alone), suggesting LDL oxidation may occur quickly, implicating ox LDL in altering normal endothelial function. Another study by Motoyama *et al* showed similar short term effects of an antioxidant improving endothelial function in the brachial arteries of patients with known impairment of vascular endothelium [78]. Lekakis and coworkers have shown acute impairment of endothelium-mediated function in individuals who smoke cigarettes, suggesting that changes in endothelial function in brachial arteries occurs rapidly [79]. Others have found that by reducing cholesterol levels in hypercholesterolemic patients that an improvement in endothelium-dependent vasodilation is seen in only four weeks [72].

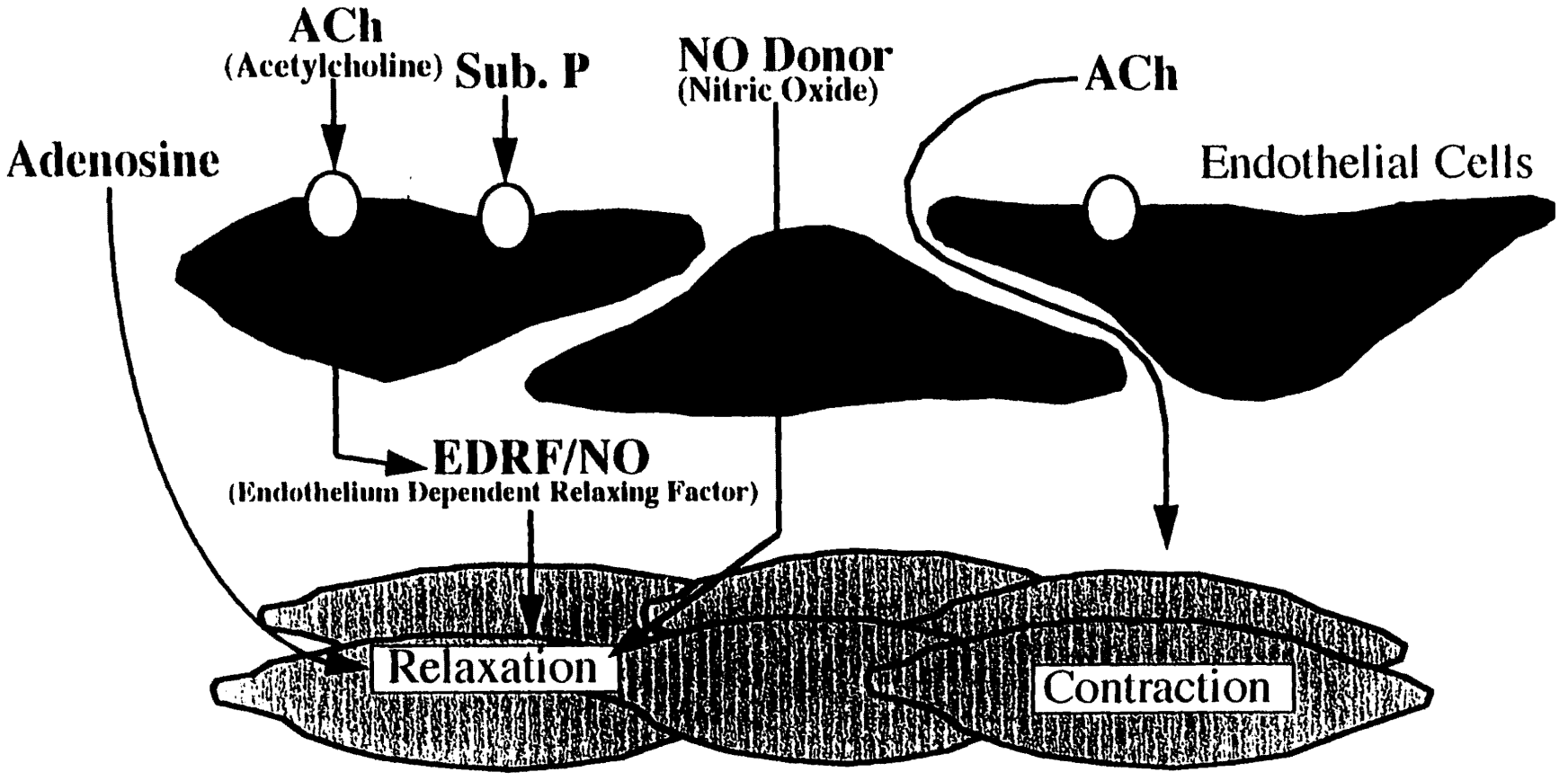
RATIONALE

It is known that the endothelium plays a major role in contributing to the coronary artery's ability to vasodilate and maintain normal vascular tone. A variety of pharmacologic agents are able to elicit nitric oxide production from an intact endothelium and to induce smooth muscle relaxation and consequent vasodilation. Other pharmacologic agents cause vascular smooth muscle relaxation through direct actions on the vascular smooth muscle and do not require the presence of an intact endothelium for their vasodilatory effects (Figure 1). The condition of hypercholesterolemia has been shown to alter normal endothelial function in both man and animal studies, but many questions about how the endothelium is altered and how long it takes for these changes to occur *in vivo* remain. The purpose of this study is to determine the effects of short term hypercholesterolemia on the coronary endothelium by using specific drugs to evaluate normal vascular function.

Three groups of swine were used in our studies. The first group received a normolipic diet, the second group a high cholesterol diet, and the third group a high cholesterol diet and in addition was pretreated with focal coronary abrasion to mechanically disrupt the endothelium at a predetermined site. Each group received identical intracoronary injections of two classes of drugs: endothelium-dependent vasodilators and endothelium-independent vasodilators. Substance P (SP) and acetylcholine (ACH) act through an intact endothelium via receptors that trigger the endothelium to produce nitric oxide which then diffuses to the underlying smooth muscle. Adenosine (ADO) and nitroglycerin (NTG) act directly on the vascular smooth muscle to cause vasodilation, but the precise

Figure 1

Mechanisms of Acetylcholine, Substance P, Adenosine and Nitroglycerin



mechanism of these two drugs differ: ADO allows for smooth muscle hyperpolarization and decreases intracellular calcium, while NTG is a NO donor. Changes in coronary blood flow and changes in resistance to coronary blood flow in response to each drug were measured to determine vascular reactivity under each experimental condition.

The specific aims to be accomplished based on these tests were:

1. To determine the effects of short term hypercholesterolemia, specifically high concentrations of LDL, on endothelium-mediated changes in coronary blood flow.

2. To determine the effects of short term hypercholesterolemia on the resistance to coronary blood flow, both globally and focally in the coronary arterial system, by using the aforementioned pharmacologic agents.

Both global and focal hemodynamic changes in response to each of the drug injections were examined. By denuding the coronary vascular endothelium, we were able to compare the effects of mechanical endothelial damage at a specific region of the vasculature against the effects of hypercholesterolemia on resistance to coronary blood flow over the same focal region.

EXPERIMENTAL DESIGN AND METHODS

Experimental Design

The effects of hypercholesterolemia on endothelium mediated tone were examined by using both endothelium-dependent vasodilators (SP and ACH) and endothelium-independent vasodilators (ADO and NTG). Three groups of swine were used in this study in order to allow for the investigation into both focal and global changes in endothelial function. A control (control, CTL.) group received the normolipic diet, the hyperlipidemic group (hyperlipidemic, HC) received the highly-concentrated LDL diet, and the hyperlipidemic group received the high fat diet as well as being pretreated with coronary endothelial denudation (hyperlipidemic + denuded, HC+D). Intracoronary doses of each drug were given in various concentrations, permitting the calculation of dose response curves. Dose response curves were generated for changes in coronary blood flow, global resistance to blood flow, and local resistance to coronary blood flow in response to the various drugs administered. The concentrations of the drugs given were as follows:

Acetylcholine: 0.01, 0.1, 1.0, and 2.0 μg/ml

(5.5×10^{-8} M, 5.5×10^{-7} M, 5.5×10^{-6} M, and 1.1×10^{-5} M acetylcholine)

Substance P: 1.0, 2.0, and 4.0 μg/ml

(7.4×10^{-7} M, 1.5×10^{-6} M, and 3.0×10^{-6} M substance P)

Adenosine: 1.0, 2.0, 4.0, 10.0, 20.0, and 40.0 μg/ml

(3.7×10^{-6} M, 7.5×10^{-6} M, 1.5×10^{-6} M, 3.7×10^{-5} M, 7.5×10^{-5} M, and 1.5×10^{-4} M adenosine)

Nitroglycerin: 1.0, 2.5, 5.0, and 10.0 μg/ml

(4.4×10^{-6} M, 1.1×10^{-5} M, 2.2×10^{-5} M, and 4.4×10^{-5} M nitroglycerin)

All of the values chosen were derived from previous studies performed by this laboratory and were found to be effective in the swine model. The doses of ACH and NTG are also used clinically to evaluate endothelial function in human coronary arteries. The doses of SP were chosen specifically to avoid possible endothelial sensitization as well as to avoid any possible significant long-term changes in mean arterial blood pressure due to SP's potent vasodilatory actions in peripheral blood vessels. Prior to the administration of each class of drug, a vehicle injection of the same volume as that of the drug was given. This was important in that when the data were analyzed, any artifactual changes in flow or resistance due to the fluid injection alone could be identified. Administration of each group of drugs and doses of specific drugs was randomized for each experiment.

During the course of each experiment, ventricular, LAD, and coronary sinus catheters facilitated the measurement of the appropriate pressures which permitted the determination of both focal resistance (at the site of LAD denudation) and global resistance to coronary blood flow. Coronary blood flow (ml/min/100g) was monitored by the use of a perivascular Doppler flow probe. In all of the calculations of CBF and both global and focal resistance values, flow was standardized to 100 grams of tissue perfused to normalize variations in flow due to the variation in weight of the area of the myocardium perfused. We used the following equation to calculate the resistance to global coronary blood flow:

$$\frac{\text{mean arterial blood pressure (mmHg) - coronary sinus mean pressure (mmHg)}}{\text{coronary blood flow (ml/min)}}$$

In order to examine the changes in resistance to coronary blood flow at the site of endothelial denudation (focal resistance), we used a variation of the above equation:

$$\frac{\text{mean arterial blood pressure (mmHg) - mid LAD mean pressure (mmHg)}}{\text{coronary blood flow (ml/min)}}$$

Prior to each experiment, anaerobic arterial blood gases were taken, using heparinized syringes (on ice) for pH, pO_2, and PCO_2 determination. Prior to any drug injection, a bolus of ADO was administered to ensure that our surgical manipulations did not alter normal vascular reactivity. Following the conclusion of all intracoronary injections, the heart was excised after an overdose of anesthesia. Through a small catheter inserted into the LAD, a high-carbon ink was injected to stain the area of the heart perfused by the LAD allowing weight determinations of the total heart as well as the area perfused by the LAD. Histologic preparation of the focal section of the LAD was performed to ensure the efficacy of the mechanically-induced endothelial damage to the group which received this treatment.

METHODS:

A protocol which included three groups of healthy domestic male and female swine (n=5/group), aged 3-4 months weighing 20-25 kg, was developed. The control group (**CTR**) received normal pig chow while the high cholesterol group (**HC**) and the denudation plus high cholesterol group (**HC + D**) received a formulated diet containing 2% cholesterol and 20% lard, 4000 I.U. vitamin D, and fortified with iron (to 240 mg/kg, Bioserve, Frenchtown, NJ). Each group received approximately 0.5 kg of their respective diet per day. Lipid profiles were measured in each swine on the day of the coronary artery injections.

Surgical preparations for coronary denudation:

For the HC+D group, on the day of denudation, each pig was sedated with 15 mg/kg Ketamine hydrochloride (Vetalar®, Fort Dodge Laboratories, MI) intramuscular (IM), weighed , and anesthetized with 2.5 % isoflurane inhalation (Ohmeda Carib Corp., Guayama PR). An angiocatheter (20g, Deseret Medical, UT) was inserted into a marginal ear vein for venous access. The animals were placed in dorsal recumbence on a V-tray, clipped and scrubbed ventrally on the neck, and draped to prepare an aseptic field for surgery.

During the surgical procedure, heparin sodium (Pharmacia & Upjohn Corp., MI) was administered intravenously (IV) through the ear vein at a dose of 200 units/kg of body weight, with repeated doses of 80 units/kg given every 20 minutes for the remainder of the procedure. Also, the pigs were started on an IV drip of nitroglycerin (NTG) (77 ml/hr, 0.25 mg/ml, Solopak Laboratories, IL,) and

an IV drip of lidocaine (44 ml/hr1.36 mg/ml, Abbott Laboratories, IL), both clinically derived values found to prevent coronary vasospasm and arrythmias respectively during coronary angiography.

Using aseptic technique, we made a ventral midline incision and isolated and temporarily ligated the left carotid artery. Using a 9F catheter introducer system (Catheter Sheath Introducer System, Cordis Corporation, Miami, FL), access to the lumen of the carotid artery was made by making a small incision across the wall of the artery. The incision was created by using a 14g Angiocath (Deseret Medical, UT). The fully assembled sheath system was introduced into the incision to access the artery lumen, the sheath was fastened into position with Lig-a Loops (Baxter Healthcare, IL), and the dilator and wire were removed. The catheter sheath used has a valve opening through which a guide catheter (8F, 100 cm Marathon® Guiding catheter, Baxter Healthcare Corporation, IL) was advanced under fluoroscopy into the ostium of the left anterior descending coronary artery (LAD). A steerable guide wire (Hi-Per™Flex™ Steerable Guide Wire, USCI Division, C.R. Bard, USA) was advanced angiographically through the guide catheter to the LAD with the assistance of a radiopaque contrast media (Renografin®-76, Squibb Diagnostics, NJ). A balloon angioplastic catheter (Titan 18, 3.9F proximal, 4.0 mm balloon PTCA Dilation Catheter, Cordis Corp., FL) was advanced over this guide wire and angiographically positioned in the LAD in an area free of branch vessels. The balloon was then inflated with a solution of 50% saline and 50% Renografin to 2 atm (or until firm contact was made with the wall of the LAD), withdrawn 1 to 2 cm, deflated, and then readvanced to its previous position in the LAD. This procedure was repeated five times to adequately

denude the endothelium. Contrast dye injection was used to insure that the balloon was pressed securely against the LAD wall prior to denudation. A control angiogram was taken to record the site of denudation. A post-denudation angiogram was taken to note changes in the vessel appearance and assure patency of the vessel. Once the denudation was completed, all catheters were removed, the sheath was removed, and the opening in the carotid was closed with 6-0 Prolene® monofilament polypropylene suture (Ethicon, Inc., Sommerville, NJ). The neck incision was closed with 3-0 Ethilon® monofilament nylon (Ethicon, Inc.) in the subcutaneous tissues and the skin was closed with Royal®-35W disposable skin staples (United States Surgical Corporation, OH). A post-surgical administration of 250 mg IM and 250 mg IV cefazolin sodium (Zolicef™, Solopak Laboratories, IL) was administered as an antibiotic therapy. Animals were monitored for potential post surgical complications.

After the denudation procedure, all animals remained on the atherogenic diet for a total of no less than 14 days.

Surgical preparation for experimental studies:

For the CTL, HC, and HC+D groups, on the 14th day of receiving their respective diet, each animal was sedated as described above. Through the angiocatheter in the ear vein, IV sodium pentobarbital (50 mg/kg, Sigma Chemical Co., MO) was used to achieve a plain of anesthesia. Endotrachial intubation was performed to maintain an airway. Intravenous NTG and lidocaine were also started, as well as heparin administration, as previously described. Isolation and

sheath introduction into the right carotid artery was accomplished in the same manner as previously mentioned. The right external jugular vein was also isolated and sheathed in a similarly. The right femoral artery and vein were dissected and isolated. The femoral artery received a sheath through which a 7F Mikro-tip® dual pressure transducer catheter (Millar Instruments, TX) was advanced. Under fluoroscopic guidance, this catheter went past the atrioventricular valve and was seated into the left ventricle for simultaneous measurements of aortic root and intraventricular pressures. The femoral vein was catheterized with Polyethylene 240 (Intramedic, Becton Dickenson, NJ) for the purpose of heparinization and continuous pentobarbital administration (pentobarbital given at 0.15 ml/min., syringe pump model 351, Sage Instruments, MA).

The jugular vein received a 7F Goodale-Lubin right heart catheter (Cordis Corp., FL) that was advanced under fluoroscopic guidance into the coronary sinus for the purpose of measuring coronary sinus pressure. A steerable guide wire was passed through the coronary introducer catheter and into the LAD. Using the steerable guidewire, all animals received an infusion catheter (2.5F, 125 cm Infusion Catheter, Cordis Corp., FL) that was advanced over the guidewire. Fluoroscopy and contrast dye injection was used to ensure proper positioning of the infusion catheter into the coronary osteum. The infusion catheter was placed just proximal to the site of denudation. A 2F Millar single pressure catheter/velocimeter (Millar Instruments, Houston TX) was also positioned through the coronary guide catheter and inserted into the LAD distal to the infusion catheter. At this point, NTG and lidocaine were stopped and the animal

was carefully moved from the fluoroscopy room and brought to the laboratory for experimentation.

In the laboratory, the animal was placed on a fluid-filled heating pad (Model K-20, American Hamilton, OH) on a surgical table, right laterally recumbent. The endotrachael tube was connected to a mechanical respirator (Anesthesia Ventilator, Fraser-Harlake Model 701, NY) cycling 8 to 12 ventilations per minute, with a volume of 12-15 ml/kg. The dual and single pressure transducers as well as the coronary sinus catheter and ECG leads were connected to an eight channel physiologic chart recorder (Model RS, Gould Electronics, OH). This recorder was connected to an analog-to-digital data acquisition system (CA Recorder, Data Integrated Scientific Systems, MI) used for continuous data collection and analysis. Arterial blood gases were obtained and adjustments were made to the respirator to achieve normal arterial blood gas pH (7.35-7.45), PaO_2 (95-105 mmHg), and $PaCO_2$ (32-37 mmHg) using a blood gas analyzer (Instrumentation Laboratory, Italy). Hematocrits were also taken and measured (International Microcapillary Reader, MA).

A left lateral thoracotomy was performed at the 4th intercostal space and the opening was maintained with a Finochetto rib spreader retractor. Heat cautery (300 watt, Geiger Instrument Co., PA) was used to control hemostasis. With the left lung pushed aside, an opening was made in the pericardium, sparing the phrenic nerve and all significant pericardial vessels. The left auricular appendage was retracted to gain access to the LAD.

The LAD was then carefully isolated and dissected from the epicardium so as to allow the placement of a Doppler flow probe (Transonic Systems, Inc. Ithica, NY). For the HC+D group, this was placed proximal to the site of the prior

denudation . Isolation of the vessel and placement of the probe was assisted by viewing the angiograms taken during the denudation process for the HC+D group. Lidocaine (Abbott Laboratories, IL) was applied topically to minimize vasospasm. After the LAD isolation and following placement of the flow probe, the opening in the chest wall was covered with plastic film (Saran Wrap™, Dow Chemical Co., NJ) and the preparation was allowed to stabilize for 20 minutes prior to any intracoronary injections. Once the preparation was stabilized, intracoronary injections of acetylcholine (0.01, 0.1, 1, and 2μg/ml, Sigma® Chemical Co.), substance P (1, 2, and 4 μg/ml, Sigma® Chemical Co., MO), adenosine (1, 2, 4, 10, 20, and 40 μg/ml, Sigma® Chemical Co., MO), and nitroglycerin (1, 2.5, 5, and 10 μg/ml, Solopak Laboratories, IL) were administered. After all injections, the heart was excised and placed in a warm water bath (37°C) and perfused with 10% buffered formalin (Sigma® Chemical Co., MO) at 100 mmHg using a Baumanometer (W.A. Baum Company, Inc., NY) for several minutes. The heart was then placed into the buffered formalin overnight. The following day, the LAD was removed and taken to the Pathology Department at USUHS where routine hematoxylin and eosin slides and Verhoeff's Elastic Van Gieson stain for elastin were made (chemicals from Sigma® Chemical Co., MO). Photographs of slides were made using a camera-mounted light microscope (Zeiss, West Germany) and Ektachrome 64T film (Eastman Kodak Co., NY).

INTRACORONARY DRUG INJECTIONS

Description of Dose Response Curves

In Figure 2, a sample response curve to a single injection of a vasoconstrictive drug is depicted. The injection of a drug which would cause vascular smooth muscle contraction initially creates a "trough", or a period of reduced blood flow due to the reduction in vessel lumen diameter. As the drug is either degraded or washed out of the vascular bed which is being analyzed, a "hyperemic area" develops in response to the period of reduced blood flow. The increase in blood flow in the vessel is proportional to the period of time the bed perfused by the constricted vessel was ischemic. The accumulation of vasoactive metabolites (*i.e.* adenosine and hydrogen or potassium ions) increases during an ischemic episode as the time spent in ischemia increases. In this study, doses of ACH that were found to be vasoconstrictive were analyzed by comparing the changes from baseline in both the "trough" and the "hyperemic" response.

Substance P in the intact endothelium has potent vasodilatory effects, thus doses were selected to avoid long-term systemic effects of this drug. SP has constrictor effects in vascular smooth muscle, but these effects are normally overwhelmed by the vasodilation caused by NO production. Increases in flow from baseline were measured with ADO and NTG, as these drugs do not have any vasoconstrictive effects in the vasculature.

Changes in resistance to the various drugs administered were also analyzed by using response curves. As seen in Figure 3, the response curve generated is a mirror image of that produced in Figure 2. As blood flow is

Figure 2

Flow Response Curve

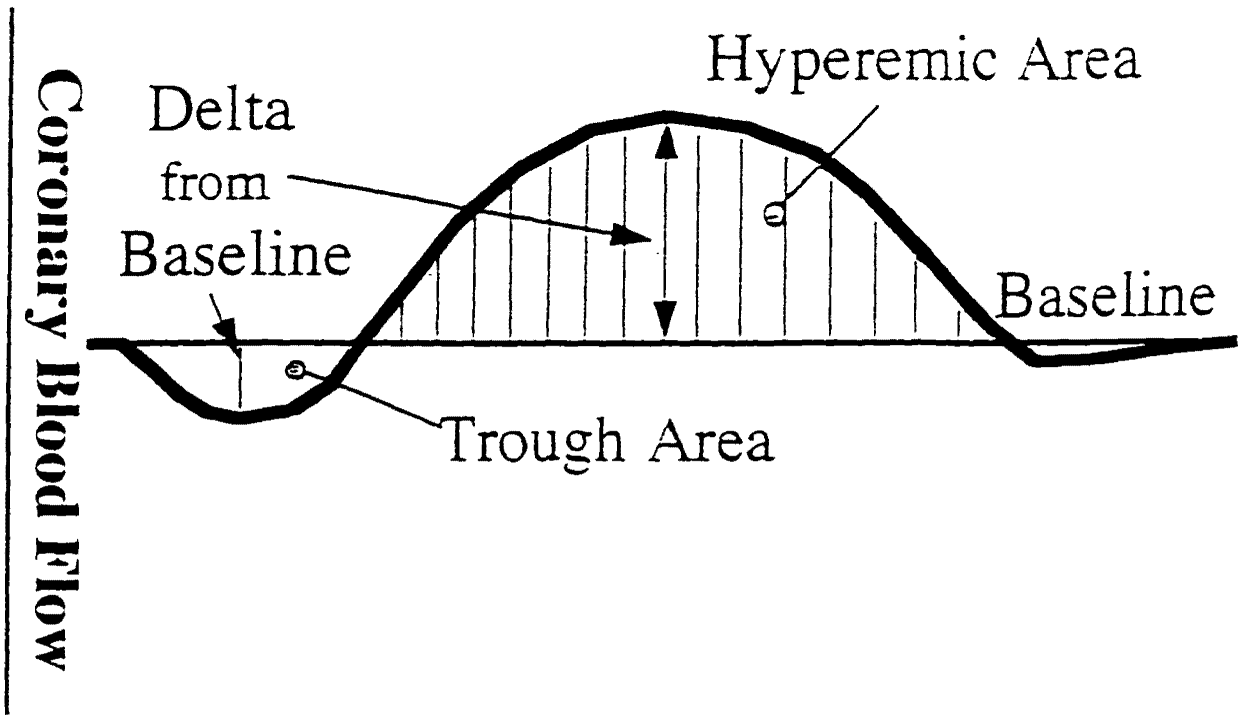
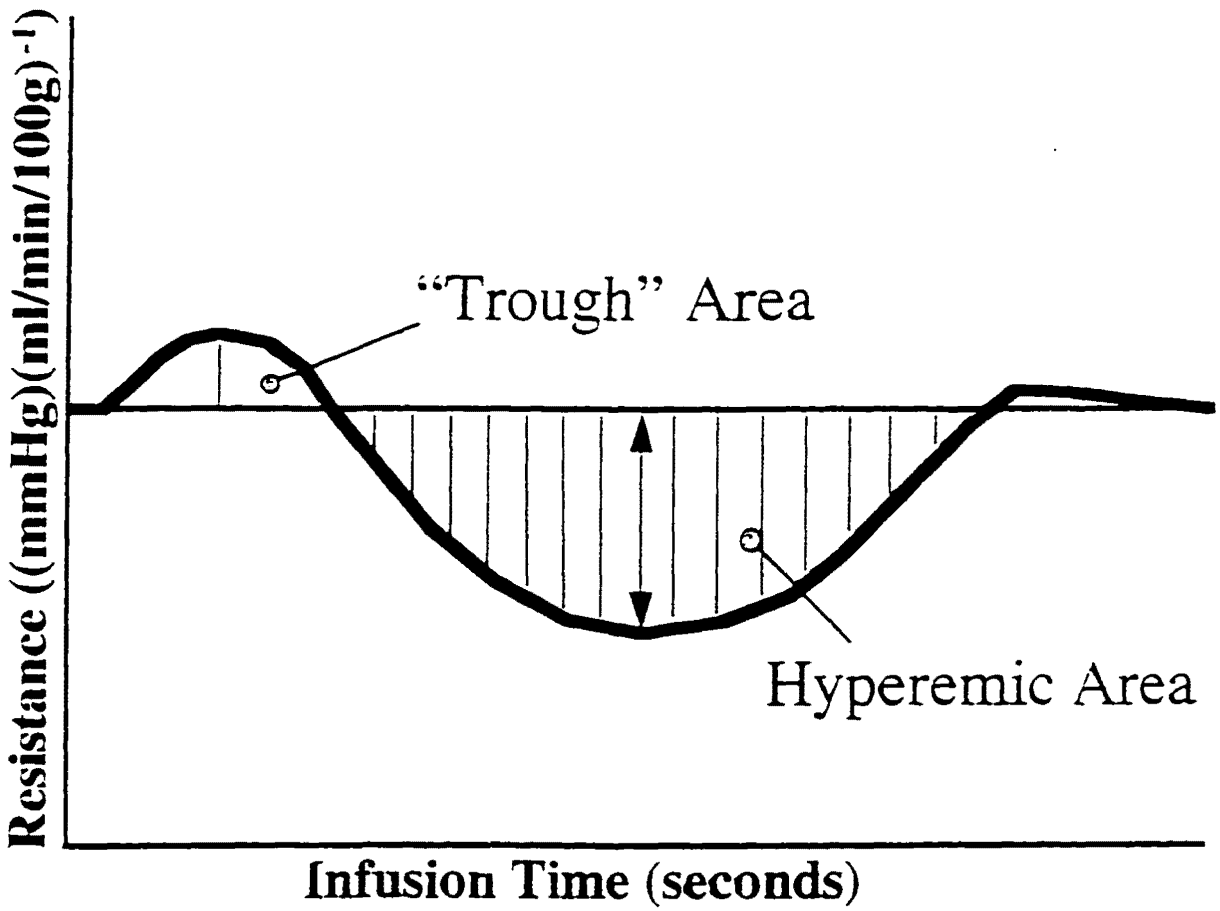


Figure 3

Resistance Response Curve



restricted by a narrowing lumen upon the administration of a vasoconstrictive drug, resistance to blood flow increases. As the drug is removed from the circulation, the vasoactive metabolites increase lumen diameter and reduce the resistance to blood flow until the hyperemic response is terminated and baseline values return.

Measurement of focal resistance was recorded between the coronary ostium and at a site distal to the area of angiographically determined coronary abrasion in the HC+D group. The LAD pressure transducer was carefully placed into the LAD under angiographic guidance to ensure the proper location distal to the site of previous abrasion (or its correlate in the CTL and HC groups) was established. In the CTL and the HC groups, focal resistance was recorded between the coronary ostium and at the same distal site in the LAD where the LAD pressure transducer was placed in the HC+D group. Changes in resistance were measured between this small portion of the coronary vascular bed. The process of endothelial denudation permitted the creation of an area of known mechanically-induced (and histologically verified) vascular endothelial damage. The hypothesis could be tested further by comparing the "focal" resistance changes between two fixed points, where verifiable endothelial damage has occurred (HC+D) and hypothesized changes might have occurred (HC).

Only the CTL and HC groups were used for coronary blood flow and global resistance comparisons while the HC+D group was used specifically for the focal resistance analysis. This was due to the fact that the HC+D group went through the denudation process prior to coronary blood flow analysis, while the HC and CTL groups did not

STATISTICAL ANALYSIS

Data were analyzed by Student's T tests (parametric) and Mann-Whitney U tests (nonparametric) when comparing the CTL group to the HC group for both coronary blood flow and global resistance to coronary blood flow. When analyzing changes in focal resistance, analysis of variance (ANOVA) tests were used to compare all three groups. Linear regression analysis was used to examine the relationship between increasing cholesterol levels to increases in blood pressure. Statistical significance was set at the $P \leq 0.05$ level.

RESULTS

Prior to each experiment, arterial blood samples were collected and analyzed for pH and oxygen and carbon dioxide partial pressures (Table 1). Hematocrits were also measured to ensure proper perivascular flow probe function.

Development of a Hyperlipidemic Model

The development of our model of hypercholesterolemia was critical to the study and was monitored by measuring serum cholesterol in all of the groups. As illustrated in Table 2, the total cholesterol, LDL and HDL concentrations were successfully increased above CTL in both the HC and the HC+D groups. Figure 4 illustrates this increase in total cholesterol in these high cholesterol groups as compared to CTL. The increase in total cholesterol levels was due to the significant increase in LDL concentration above that seen in the CTL group (Figure 5). Only the HC+D group showed an increase in HDL concentration above the CTL group.

TABLE 1

Arterial Blood Gas Data (n=5/group). All values are means±SEM

Protocol	arterial pH	arterial O_2 (mmHg)	arterial CO2 (mmHg)	Arterial Hct.
Control	7.44±0.022	98.0±5.7	40.4±1.6	30.6±0.87
Hyperlipidemic	7.54±0.018	107.4±5.3	32.4±1.9	31.6±1.5
Hyperlipidemic + Denuded	7.49±0.014	121.0±6.5	36±2.0	31.0±1.4

Table 2

Cholesterol Data (n=5/group). All values are means±SEM

Protocol	Total Cholesterol(mg/dl)	[LDL] (mg/dl)	[HDL] (mg/dl)
Control	73.6±9.9	38.0±10.3	35.6±6.3
Hyperlipidemic	164.0±23.0 *	116.2±22.9 *	47.8±1.2
Hyperlipidemic + Denuded	339.4±53.7 *†	283.4±56.6 *†	56.0±3.9 *

* = statistically different than control
† = statistically different than hyperlipidemic

Figure 4

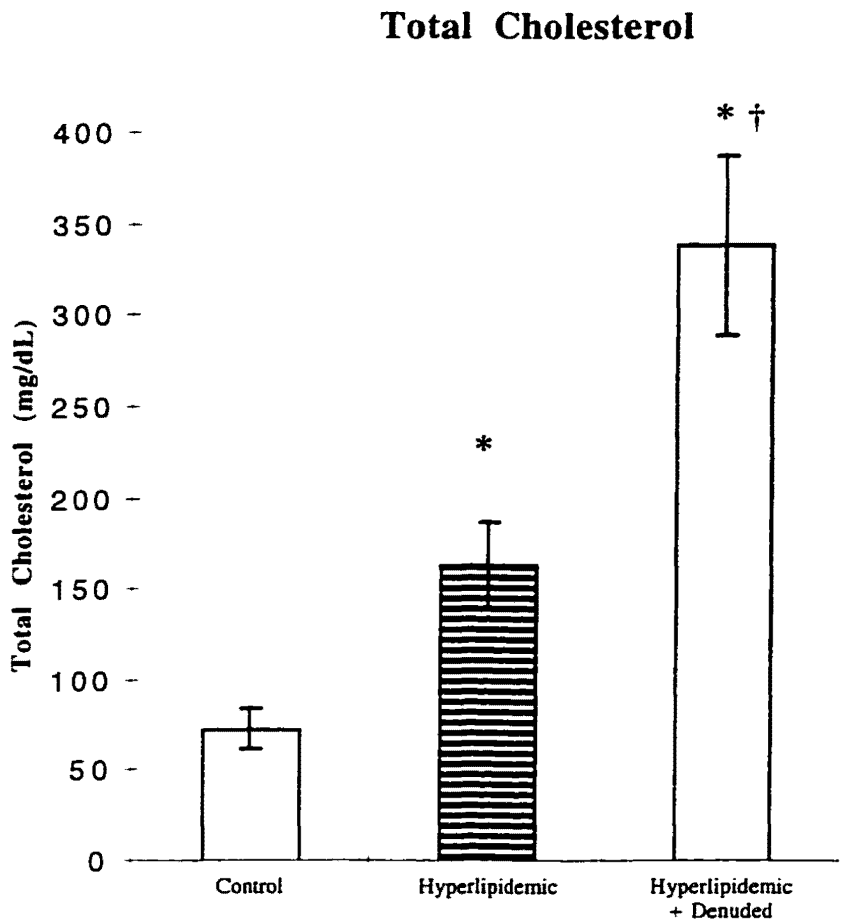


Figure 4. Total cholesterol (mg/dL) is plotted for each group. (*) represents a statistical difference from control, $p \leq 0.05$. (†) represents a stastical difference from the hyperlipidemic group, $p \leq 0.05$. The number of animals per group was Control (5), Hyperlipidemic (5), and Hyperlipidemic plus Denuded (5).

Figure 5

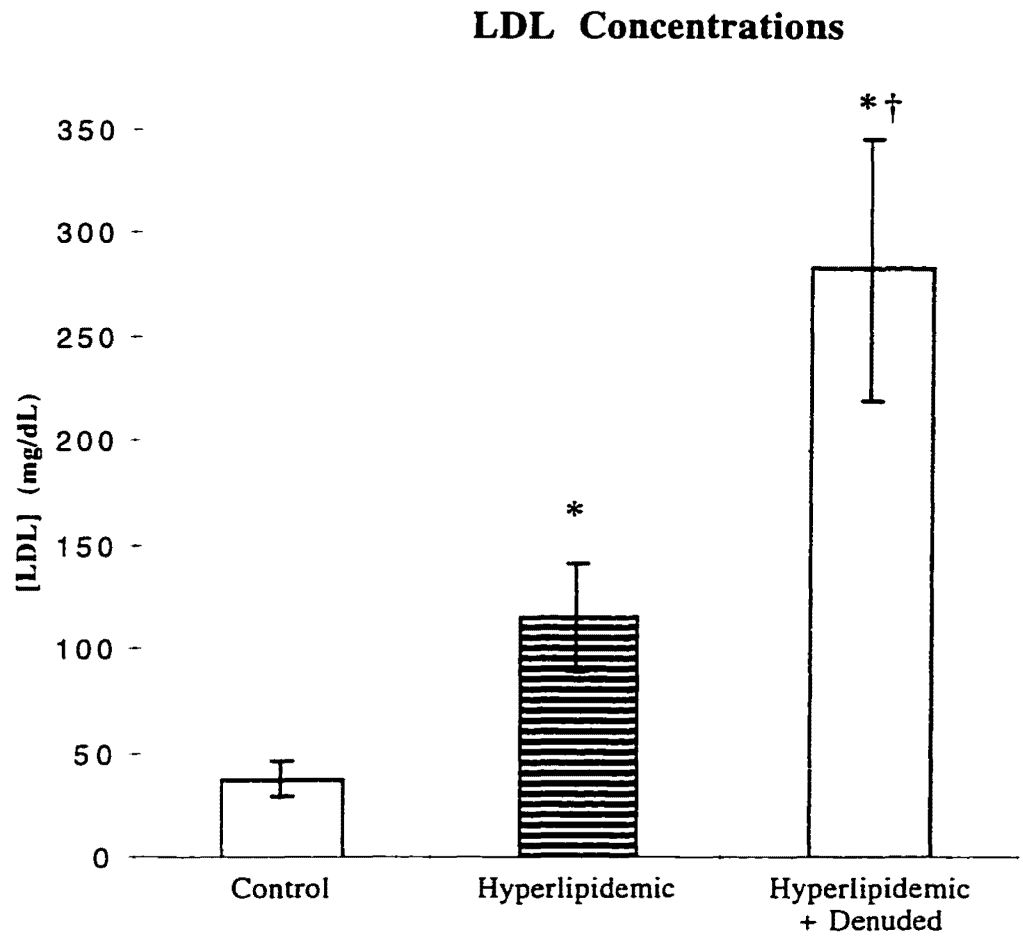


Figure 5. LDL concentrations (mg/dL) are plotted for each group. (*) represents a statistical difference from control, $p \leq 0.05$. (†) represents a statistical difference from the hyperlipidemic group, $p \leq 0.05$. The number of animals per group was Control (5), Hyperlipidemic (5), and Hyperlipidemic plus Denuded (5).

Hemodynamic Data

Table 3 shows the means of several hemodynamic parameters determined in each group. Ventricular function was measured by measuring both LVEDP, dP/dT, and PRP. Both dP/dT and LVEDP were measured and calculated by our digital data recorder by using the analog pressure inputs coming from the Millar pressure transducer in the left ventricle. PRP was calculated by multiplying systolic blood pressure by heart rate, and it is a measure of ventricular work. Only PRP in the HC group was significantly elevated above CTL. This elevation was probably due to the increases in systolic pressure in the HC group as compared to control.

Mean arterial blood pressure, systolic blood pressure, and diastolic blood pressure were all significantly increased above CTL in both the HC and HC+D groups, as illustrated in Figures 4, 5, and 6. Significant differences between groups were obtained using both ANOVA and T-Tests (when comparing CTL versus HC and HC+D combined). revealed these differences. When comparing the total cholesterol concentrations to diastolic blood pressure of the CTL and HC groups using linear regression analysis, a significant linear correlation is seen (Figure 9). Similarly, a linear relationship between rising LDL concentrations and rising MABPs was also noted in the CTL and HC groups (Figure 10). Direct arterial blood pressures were recorded prior to performing the thoracotomy in the laboratory.

Table 3

Hemodynamic Data (n=5/group). All data are represented as means±SEM.

Protocol	LVEDP (mmHg)	dP/dT (mmHg•s^{-1})	HR (beats•s^{-1})	PRP (mmHg•s^{-1})
Control	6.8±0.9	1337±167	104±6.7	9291±364
Hyperlipidemic	8.4±0.5	2017±207	121±5.9	13527±1253 *
Hyperlipidemic + denuded	8.4±1.7	1687±182	112±6.4	11550±467

Protocol	MABP (mmHg)	Diastolic BP (mmHg)	Systolic BP (mmHg)
Control	88.3±3.6	81.0±3.7	103.0±4.1
Hyperlipidemic	109.6±5.0 *	101.0±4.8 *	127.0±5.8 *
Hyperlipidemic + denuded	106.2±6.8 *	100±6.4 *	118.8±7.8 *

LVEDP = Left ventricular end-diastolic pressure

* = statistically different from control

Figure 6

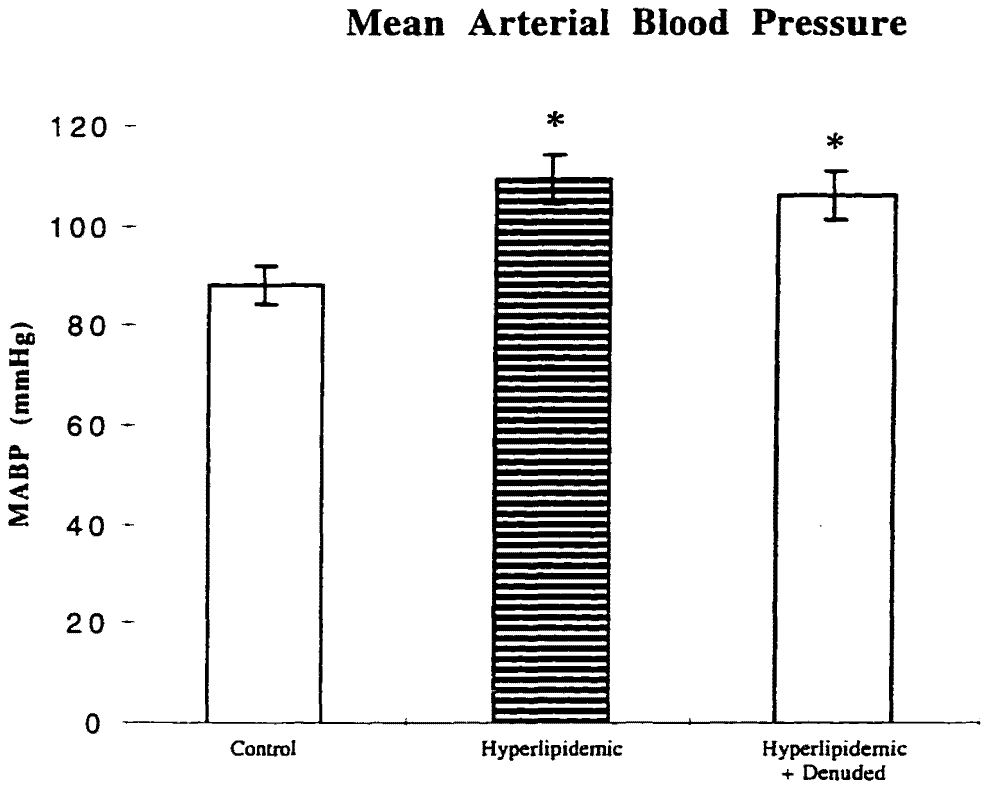


Figure 6. Mean Arterial Blood pressures (mmHg) are plotted for each group. (*) represents a significant statistical difference from control, $p \leq 0.05$. The number of animals per group was Control (5), Hyperlipidemic (5), and Hyperlipidemic plus Denuded (5).

Figure 7

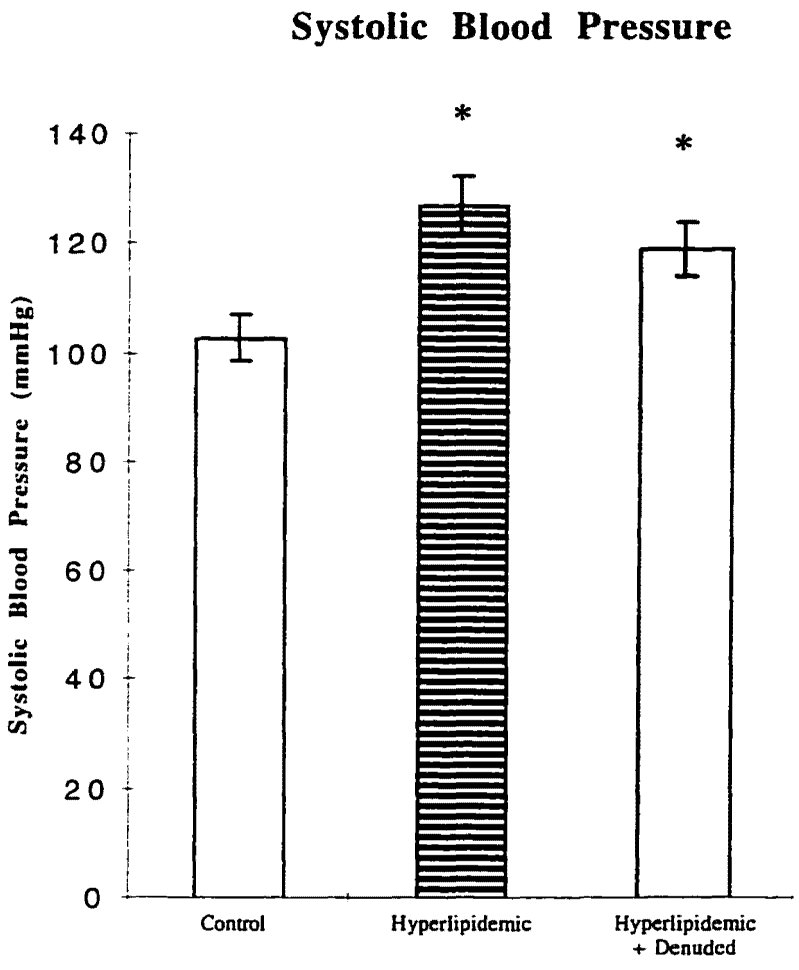


Figure 7. Systolic blood pressures (mmHg) are plotted for each group. (*) represents a significant statistical difference from control, $p \leq 0.05$. The number of animals per group was Control (5), Hyperlipidemic (5), and Hyperlipidemic plus Denuded (5).

Figure 8

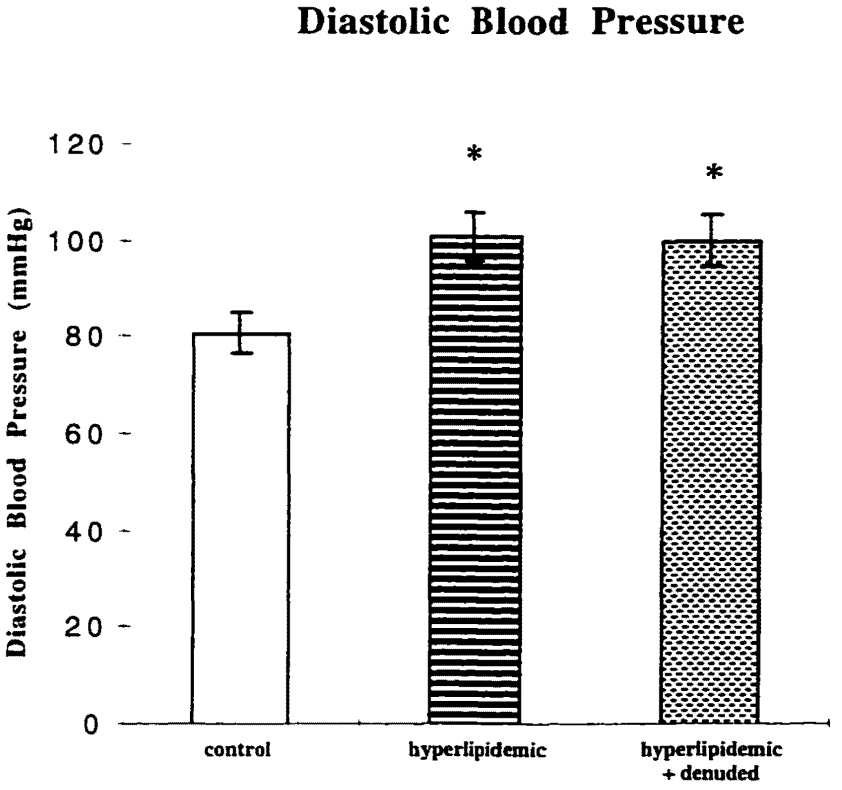


Figure 8. Diastolic blood pressures (mmHg) are plotted for each group. (*) represents a significant statistical difference from control, $p \leq 0.05$. The number of animals per group was Control (5), Hyperlipidemic (5), and Hyperlipidemic plus Denuded (5).

Figure 9

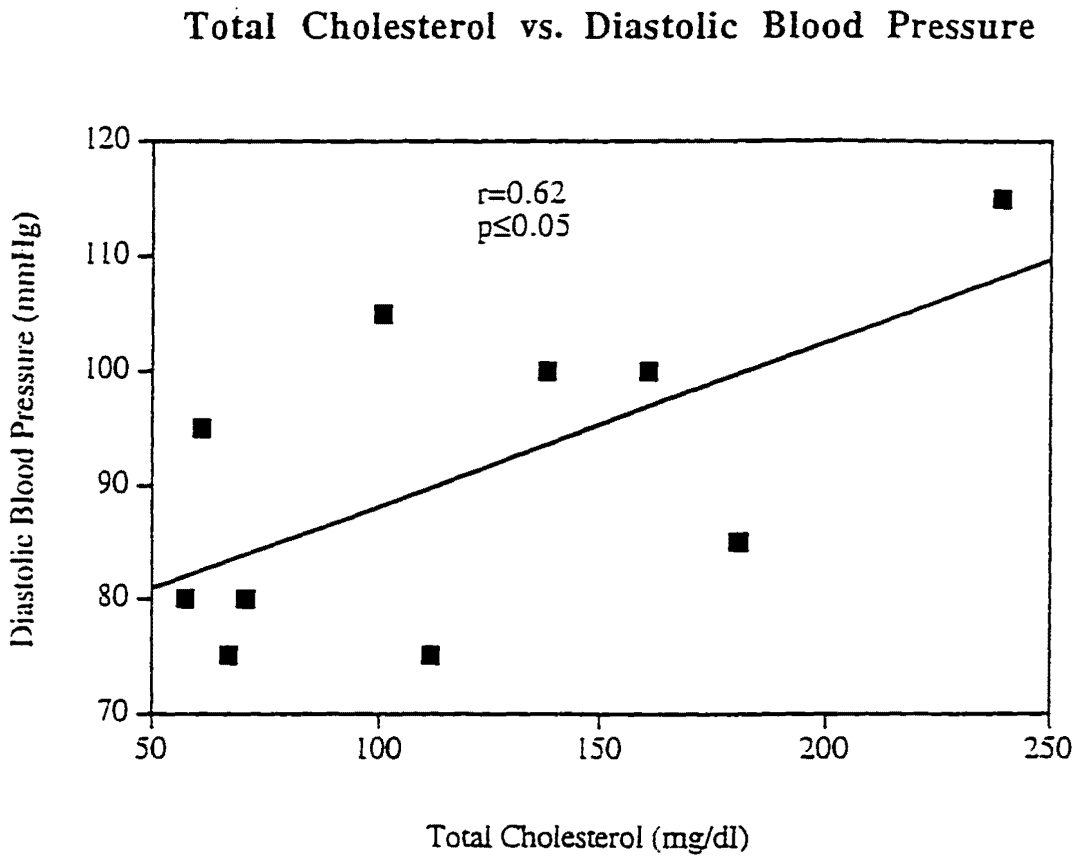


Figure 9. Total cholesterol (mg/dl) is plotted on the X-axis versus diastolic blood pressure (mmHg) on the Y-axis. A linear regression analysis was performed and was considered significant at $p \leq 0.05$. Points on the graph represent individual animals from the CTL and HC groups (n=5/group).

Figure 10

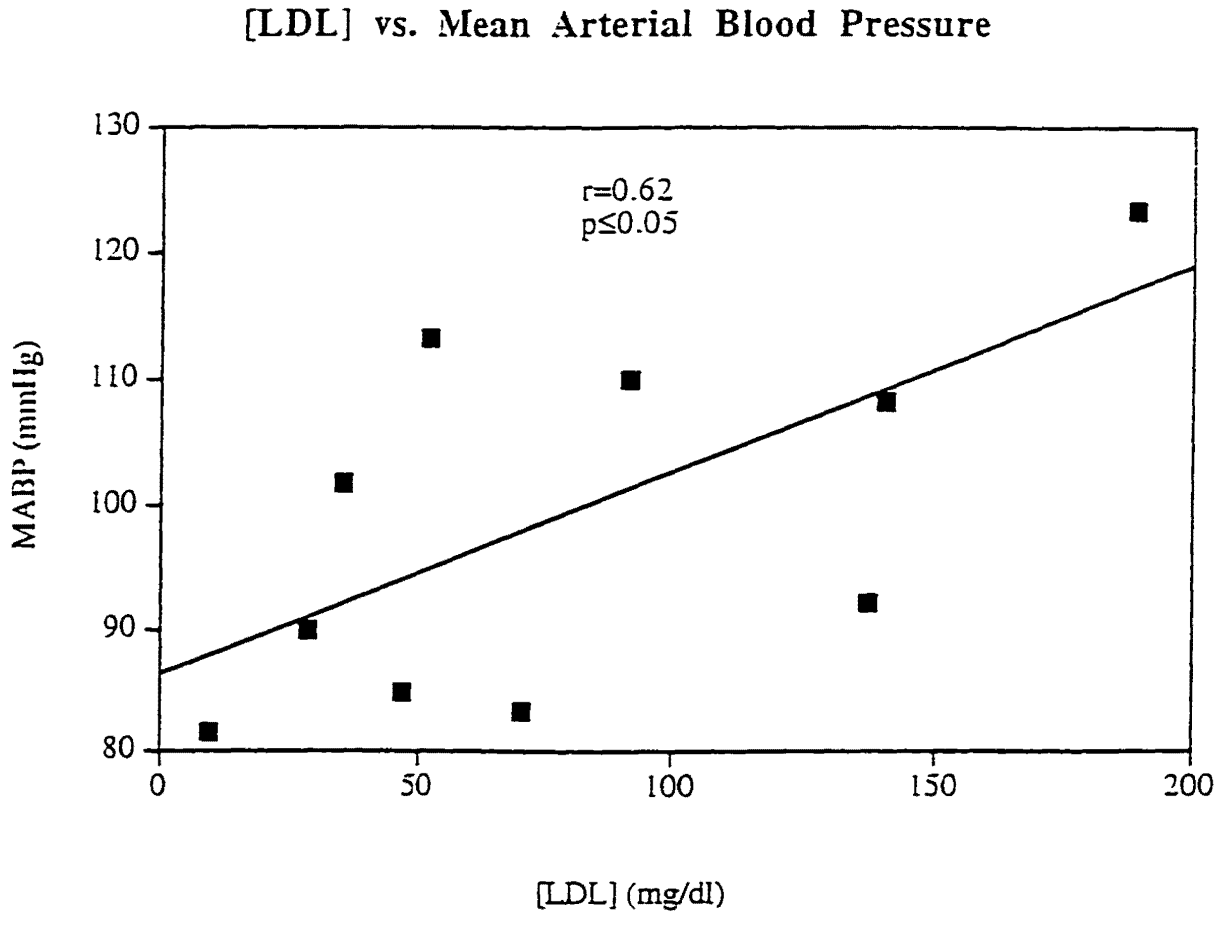


Figure 10. LDL concentration (mg/dl) is plotted on the X-axis versus mean arterial blood pressure (mmHg) on the Y-axis. A linear regression analysis was performed and was considered significant at $p \leq 0.05$. Points on the graph represent individual animals from the CTL and HC groups (n=5/group).

Changes in Coronary Blood Flow

The actions of acetylcholine (vasodilation or vasoconstriction) vary depending on the concentration of ACH injected and the integrity of the vascular endothelium. In this study, all three groups received four doses of ACH ranging from 0.001 μg/ml, which has been found to elicit coronary vasodilation in several species, to 2 μg/ml, which has been found in this and other laboratories to contract vascular smooth muscle even in vessels with intact vascular endothelium in the pig model.

The changes in coronary blood flow in response to each dose of ACH can be seen in Table 4. There was a significant difference in the hyperemic response of the CTL and HC groups to the higher concentrations of ACH. There were no differences seen in coronary blood flow between the two groups in response to the two lower concentrations of ACH. Figures 11 and 12 demonstrate graphically the decrease in coronary blood flow ("trough") in each group and the different hyperemic responses demonstrated by each group.

Changes in coronary blood flow in response to SP, NTG and ADO are tabulated in Tables 5, 6, and 7. No significant differences in coronary blood flow were found between groups using any concentration of these drugs.

Table 4

Change in Coronary Blood Flow (ml/min/100g) in Response to Intracoronary Injections of Acetylcholine (μg/ml).

Acetylcholine 0.010 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	62.8±6.8	96.4±16.0	33.6±18.6
Hyperlipidemic	88.4±10.9	108.8±12.6	26.4±10.4

Acetylcholine 0.10 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	63.8±6.4	73.4±7.4	9.6±3.4
Hyperlipidemic	89.8±10.2	99.4±14.1	9.6±8.2

Acetylcholine 1.0 μg/ml

Protocol	Baseline	Trough Change (below baseline)	Hyperemic Change (above baseline)
Control	62.2±6.7	-37.2±3.1	25.0±6.5
Hyperlipidemic	88.2±10.8	-49.1±7.3	47.4±12.6 *

Acetylcholine 2.0 μg/ml

Protocol	Baseline	Trough Change (below baseline)	Hyperemic Change (above baseline)
Control	61.6±6.3	-56.3±5.0	29.7±4.9
Hyperlipidemic	90.5±11.29	-77.5±7.2	105.9±16.3 *

* = statistically different from control. P≤0.05
All data are represented as means±SEM (n=5/group)

Figure 11

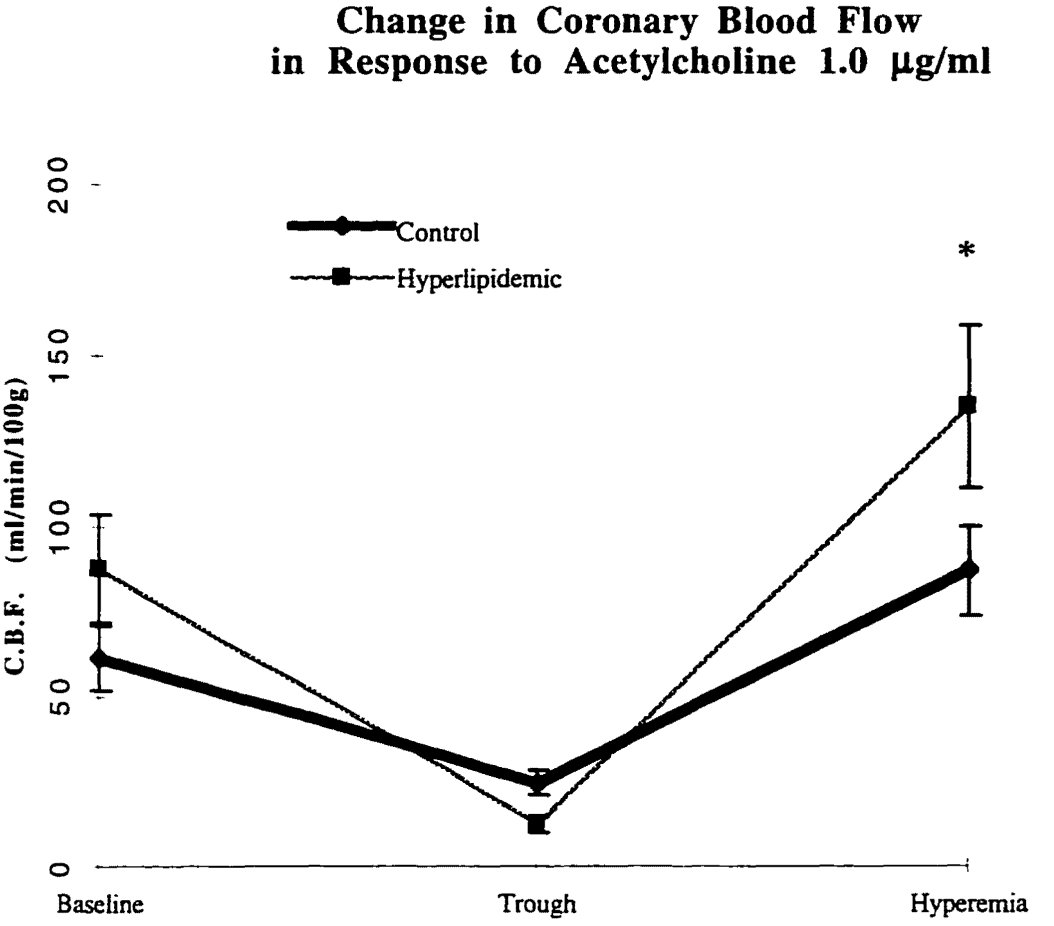


Figure 11. The changes in coronary blood flow from baseline in response to intracoronary ACH 1.0 µg/ml are plotted at baseline, during the trough, and during the hyperemic response initiated by the trough for both CTL and HC groups. (*) indicates a significant difference from control, $p \leq 0.05$. The number of animals per group was CTL (5) and HC (5).

Figure 12

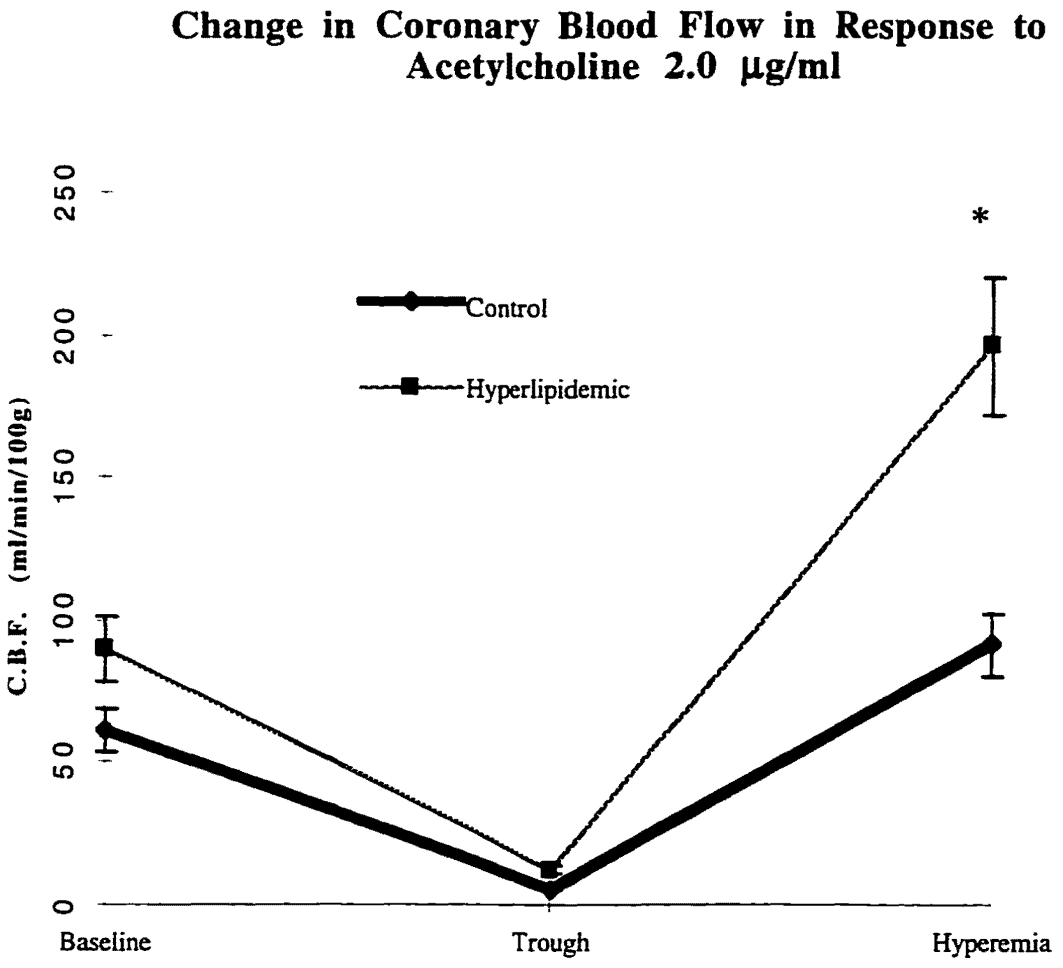


Figure 12. The changes in coronary blood flow from baseline in response to intracoronary ACH 2.0 µg/ml are plotted at baseline, during the trough, and during the hyperemic response (initiated by the trough) for both CTL and HC groups. (*) indicates a significant difference from control, $p \leq 0.05$. The number of animals per group was CTL (5) and HC (5).

Table 5

Change in Coronary Blood Flow (ml/min/100g) in Response to Intracoronary Injections of Substance P (μg/ml).

Substance P 1.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	61.6±7.3	94.6±12.6	37.0±10.0
Hyperlipidemic	76.3±9.5	110.5±14.1	34.3±18.8

Substance P 2.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	64.0±7.4	109.2±11.5	45.2±11.0
Hyperlipidemic	80.8±11.7	148±16.8	62.8±8.7

Substance P 4.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	61.0±6.9	105.2±7.8	44.2±10.8
Hyperlipidemic	87.2±4.1	123.0±8.4	35.4±7.8

All data are represented as means±SEM (n=5/group)

Table 6

Change in Coronary Blood Flow (ml/min/100g) in Response to Intracoronary Injections of Nitroglycerin (μg/ml).

Nitroglycerin 1.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	60.6±7.9	74.6±8.8	14.0±3.3
Hyperlipidemic	88.6±12.5	86.8±11.2	16.2±3.1

Nitroglycerin 2.5 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	62.8±8.3	84.0±9.7	21.1±3.2
Hyperlipidemic	87.4±11.0	116.4±9.7	29±3.3

Nitroglycerin 5.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	62.0±7.8	100.2±14.1	38.2±11.2
Hyperlipidemic	88.2±12.0	131.2±13.4	43.0±8.3

Nitroglycerin 10.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	62.6±8.6	114.8±11.1	52.2±17.6
Hyperlipidemic	88.8±11.6	120.4±26.8	51.2±14.3

All data are represented as means±SEM (n=5/group)

Table 7

Change in Coronary Blood Flow (ml/min/100g) in Response to Intracoronary Injections of Adenosine (μg/ml).

Adenosine 1.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	66.8±7.8	99.6±17.6	32.8±13.4
Hyperlipidemic	85.8±10.9	103.0±11.7	17.2±5.0

Adenosine 2.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	70.4±10.4	118.2±22.6	60.6±17.0
Hyperlipidemic	86.6±11.4	120.0±18.3	33.4±9.9

Adenosine 4.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	63.6±8.0	136.6±23.8	73.0±21.0
Hyperlipidemic	78.5±10.4	149.0±33.5	70.5±29.6

All data are represented as means±SEM (n=5/group)

Change in Coronary Blood Flow (ml/min/100g) in Response to Intracoronary Injections of Adenosine (μg/ml).

(Table 7 continued)

Adenosine 10.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	63.2±6.5	164.2±27.1	101.0±23.7
Hyperlipidemic	85.0±10.2	197.2±20.8	112.2±15.5

Adenosine 20.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	65.4±7.0	185.4±28.9	120.0±24.8
Hyperlipidemic	85.8±10.9	232.2±25.4	146.4±19.4

Adenosine 40.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	57.3±6.3	205.8±44.9	148.5±39.6
Hyperlipidemic	85.4±11.5	251.4±34.2	166.0±26.3

All data are represented as means±SEM (n=5/group)

Changes in Global Coronary Resistance

The changes in global coronary resistance were measured in response to each of the drugs at every concentration administered. The changes in resistance to each drug are shown in Tables 8, 9, 10, and 11. These changes were measured 5 to 7 seconds after each drug was injected to avoid any changes in resistance due to saline artifact. Acetylcholine 0.01 μg/ml induced changes in global resistance that were significantly different between the CTL and HC groups, as demonstrated in Figure 13. The CTL group shows a decrease in resistance to blood flow in response to the lowest concentration of ACH given (0.01μg/ml) while the HC group shows an increase in resistance at the same dose.

There appeared to be no changes between CTL and HC in global resistance to coronary blood flow when using the other concentrations of ACH or any dose of SP, NTG, or ADO.

Table 8

Global Resistance Change (mmHg·[ml/min/100g]$^{-1}$) in Response to Intracoronary Injections of Acetylcholine (μg/ml).

Acetylcholine 0.010 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	1.23±0.14	0.82±0.15	-0.41±0.24
Hyperlipidemic	1.08±0.20	1.13±0.22	0.041±0.028 *

Acetylcholine 0.10 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	1.22±0.16	1.09±0.14	-0.13±0.065
Hyperlipidemic	1.09±0.19	1.22±0.38	0.17±0.22

Acetylcholine 1.0 μg/ml

Protocol	Baseline	Trough Difference	Hyperemic Difference
Control	1.28±0.18	2.41±1.62	0.39±0.46
Hyperlipidemic	1.05±0.16	1.46±0.60	-0.41±0.11

Acetylcholine 2.0 μg/ml

Protocol	Baseline	Trough Difference	Hyperemic Difference
Control	1.22±0.11	9.35±4.27	-0.49±0.12
Hyperlipidemic	1.07±0.22	9.81±2.88	-0.45±0.31

* = statistically different from control, $p \leq 0.05$
All data are represented as means±SEM

Figure 13

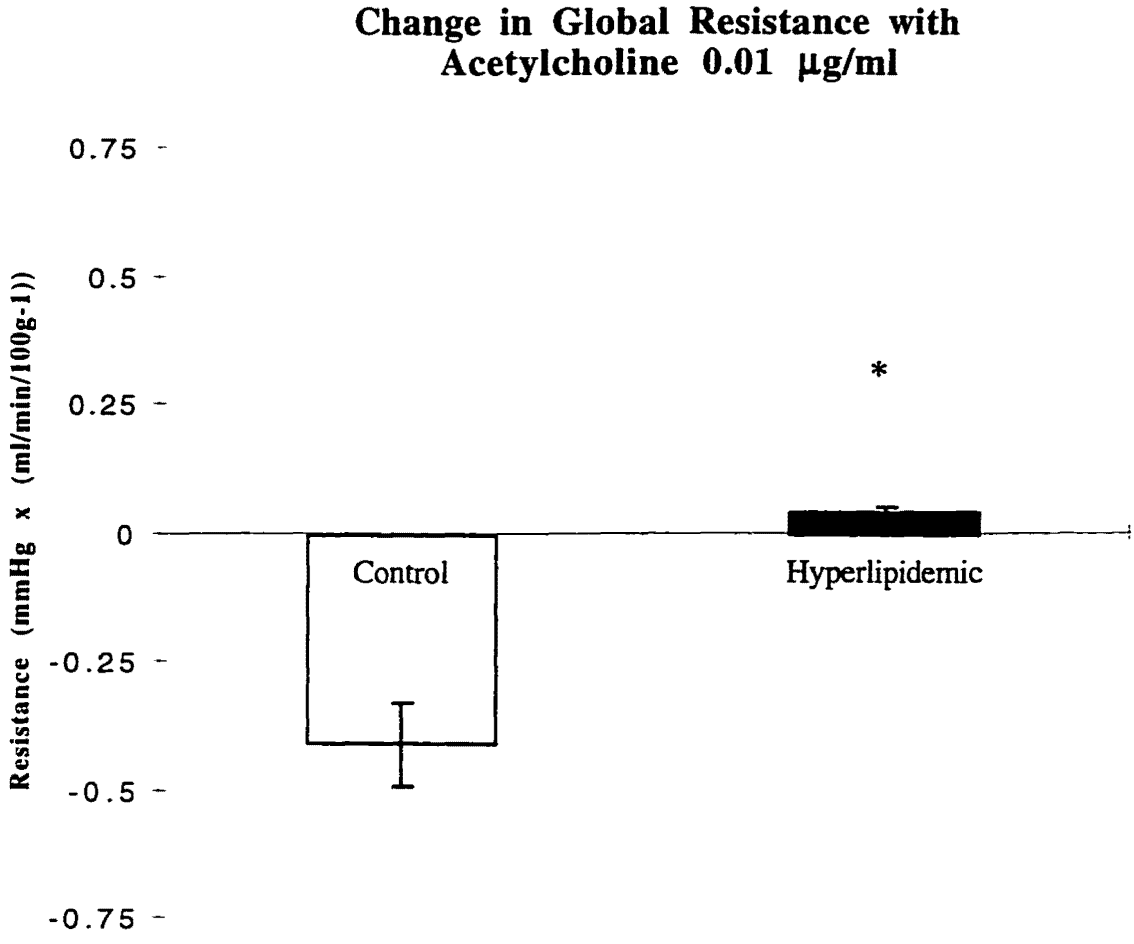


Figure 13. The change in global coronary resistance in response to intracoronary ACH 0.01 µg/ml is plotted for CTL and for HC. (*) represents a significant difference from control, $p \leq 0.05$. The number of animals in each group is CTL (5) and HC (5).

Table 9

Global Resistance Change (mmHg·[ml/min/100g]$^{-1}$) in Response to Intracoronary Injections of Substance P (μg/ml).

Substance P 1.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	1.31±0.58	0.87±0.39	-0.43±0.19
Hyperlipidemic	1.12±0.50	1.00±.45	-0.077±0.035

Substance P 2.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	1.25±0.56	0.83±0.37	-0.42±0.19
Hyperlipidemic	1.18±0.53	0.84±0.37	-0.35±0.15

Substance P 4.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	1.30±0.58	0.87±0.39	-0.44±0.20
Hyperlipidemic	0.97±0.44	0.75±0.33	-0.23±0.10

All data are represented as means±SEM

Table 10

Global Resistance Change (mmHg·[ml/min/100g]$^{-1}$) in Response to Intracoronary Injections of Nitroglycerin (μg/ml).

Nitroglycerin 1.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	1.35±0.18	1.16±0.18	-0.24±0.031
Hyperlipidemic	1.06±0.16	0.94±0.11	-0.11±0.08

Nitroglycerin 2.5 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	1.32±0.18	1.08±0.14	-0.24±0.053
Hyperlipidemic	1.07±0.15	0.87±0.10	-0.20±0.059

Nitroglycerin 5.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	1.27±0.16	0.98±0.076	-0.24±0.053
Hyperlipidemic	1.08±0.19	0.97±0.13	-0.11±0.11

Nitroglycerin 10.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	1.26±0.17	0.94±0.10	-0.33±0.07
Hyperlipidemic	1.10±0.18	0.86±0.18	-0.24±0.15

All data are represented as means±SEM

Table 11

Global Resistance Change (mmHg·[ml/min/100g]$^{-1}$) in Response to Intracoronary Injections of Adenosine (μg/ml).

Adenosine 1.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	1.15±0.20	0.65±0.063	-0.51±0.20
Hyperlipidemic	1.04±0.14	0.68±0.10	-0.36±0.10

Adenosine 2.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	1.21±0.19	0.56±0.051	-0.64±0.19
Hyperlipidemic	1.04±0.12	0.50±0.14	-0.42±0.083

Adenosine 4.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	1.19±0.19	0.58±0.11	-0.60±0.12
Hyperlipidemic	1.07±0.15	0.53±0.096	-0.53±0.14

All data are represented as means±SEM

Global Resistance Change (mmHg·[ml/min/100g]$^{-1}$) in Response to Intracoronary Injections of Adenosine (μg/ml).

(Table 11 continued)

Adenosine 10.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	1.19±0.14	0.46±0.079	-0.73±0.092
Hyperlipidemic	1.11±0.17	0.46±0.063	-0.74±0.13

Adenosine 20.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	1.15±0.14	0.39±0.060	-0.76±0.090
Hyperlipidemic	1.04±0.14	0.38±0.050	-0.66±0.11

Adenosine 40.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	1.28±0.17	0.38±0.08	-1.05±0.20
Hyperlipidemic	1.08±0.17	0.38±0.059	-0.70±0.14

All data are represented as means±SEM

Changes in Focal Coronary Resistance

Measurement of focal resistance was recorded between the coronary ostium and at a site distal to the area of angiographically determined coronary abrasion in the HC+D group. The LAD pressure transducer was carefully placed into the LAD under angiographic guidance to ensure its proper positioning distal to the site of previous abrasion (or its correlate in the CTL and HC groups). We were able to measure the drug-induced changes in resistance in this small portion of the coronary vascular bed. The process of endothelial denudation allowed us to create an area of known mechanically-induced (and histologically verified) vascular endothelial damage. We could then further test our hypothesis by comparing the "focal" resistance changes between two fixed points, where verifiable endothelial damage had occurred (HC+D) and hypothesized changes might have occurred (HC).

Changes in focal resistance in response to ACH, SP and NTG are listed in Tables 12, 13, and 14. Focal resistance changes for ADO were not calculated since the vasodilatory effects of ADO are caused due to changes in the microcirculation. Thus any changes in focal resistance with ADO in the small focal area might not necessarily be due to changes in the vessel wall at the area of investigation. NTG is known to be able to dilate the larger arterial conductance vessels of the coronary circulation, thus of the two endothelium independent vasodilators, it would have been expected to have the greatest effects at the focal site.

Both ACH and SP induced significant changes in focal resistance in the HC and HC+D compared to the CTL group. For ACH 0.01 and 0.10 μg/ml doses,

an increase in focal resistance is demonstrated in the HC and HC+D groups, while a decrease in resistance is seen over the same focal area in response to these ACH injections (Figures 14 and 15). The lowest dose of SP shows the same finding - an increase in focal resistance for both lipid groups, while CTL dilates over the same focal area (Figure 16). There were no statistical differences between HC and HC+D for any dose of SP, ACH or NTG.

Table 12

Focal Resistance Change (mmHg·[ml/min/100g]$^{-1}$) in Response to Intracoronary Injections of Acetylcholine (μg/ml).

Acetylcholine 0.010 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	0.040±0.014	0.0088±0.004	-0.031±0.01
Hyperlipidemic	0.046±0.019	0.076±0.03	0.013±0.003 *
Hyperlipidemic + Denuded	0.029±0.0089	0.046±0.01	0.018±0.004 *

Acetylcholine 0.10 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	0.026±0.011	0.014±0.009	-0.011±0.0046
Hyperlipidemic	0.052±0.018	0.062±0.018	0.007±0.003 *
Hyperlipidemic + Denuded	0.037±0.016	0.040±0.015	0.003±0.007 *

Acetylcholine 1.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	0.029±0.0093	0.043±0.012	0.014±0.0065
Hyperlipidemic	0.056±0.020	0.079±0.015	0.024±0.0076
Hyperlipidemic + Denuded	0.033±0.010	0.093±0.020	0.06±0.017

Acetylcholine 2.0 μg/ml

Protocol	Baseline	Trough Change	Hyperemic Change
Control	0.023±0.0039	0.46±0.24	0-0.018±0.0059
Hyperlipidemic	0.059±0.025	0.13±.044	-0.018±0.010
Hyperlipidemic + Denuded	0.017±0.013	0.21±0.055	-0.0046±0.0033

* = statistically different from control, $p \leq 0.05$
All data are represented as means±SEM (n=5/group)

Figure 14

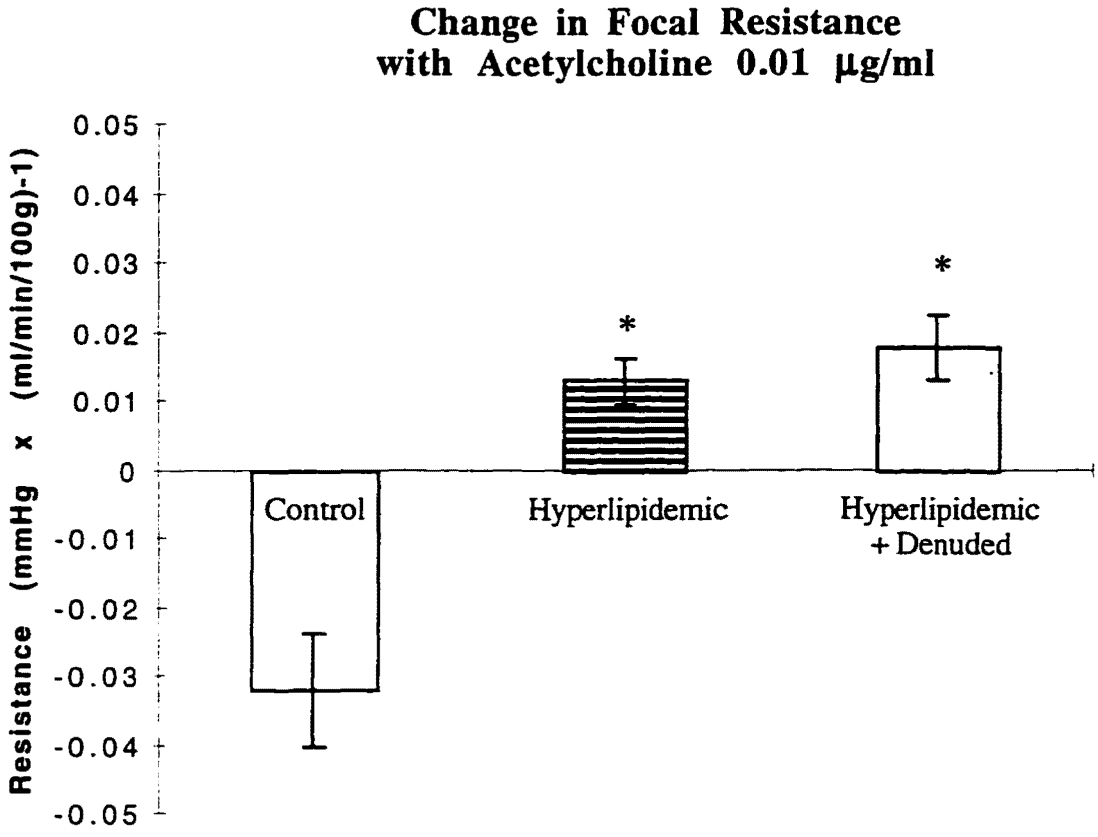


Figure 14. Change in focal coronary resistance from baseline in response to intracoronary injection of ACH 0.01 µg/ml. (*) represents a significant difference from control, $p \leq 0.05$. The number of animals in each group were CTL (5), HC (5), and HC+D (5).

Figure 15

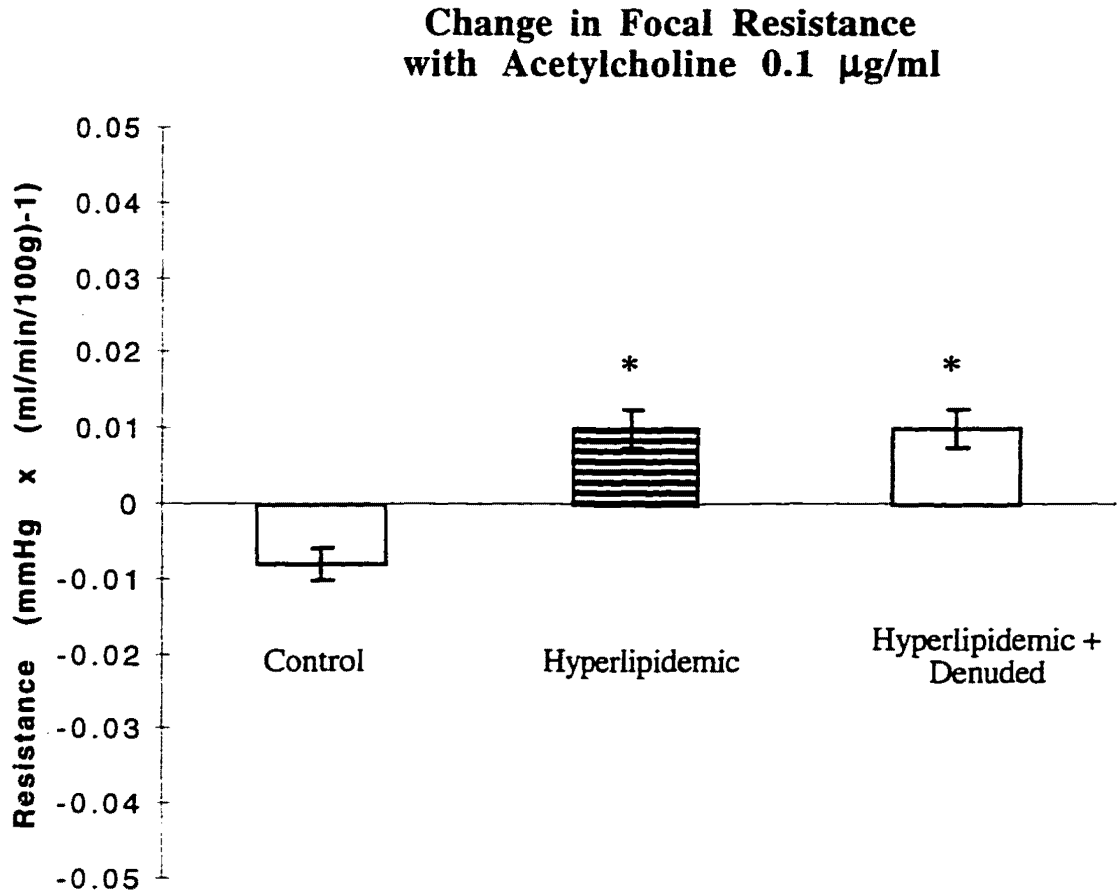


Figure 15. Change in focal resistance in response to intracoronary ACH 0.10 µg/ml. (*) represents a significant difference from control, $p \leq 0.05$. The number of animals in each group is CTL (5), HC (5), and HC+D (5).

Table 13

Focal Resistance Change (mmHg·[ml/min/100g]$^{-1}$) in Response to Intracoronary Injections of Substance P (μg/ml).

Substance P 1.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	0.025±0.006	0.019±0.005	-0.0057±0.002
Hyperlipidemic	0.049±0.016	0.081±0.021	0.081±0.050 *
Hyperlipidemic + Denuded	0.030±0.010	0.038±0.013	0.067±0.065 *

Substance P 2.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	0.025±0.0073	0.015±0.0017	-0.017±0.0070
Hyperlipidemic	0.059±0.022	0.040±0.014	-0.019±0.0082
Hyperlipidemic + Denuded	0.051±0.022	0.084±0.022	0.095±0.073

Substance P 4.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	0.032±0.010	0.021±0.0054	-0.0083±0.0041
Hyperlipidemic	0.046±0.018	0.044±0.022	-0.0028±0.0090
Hyperlipidemic + Denuded	0.019±0.012	0.021±0.010	0.0027±0.0063

* = statistically different from control, $p \leq 0.05$
All data are represented as means±SEM (n=5/group)

Figure 16

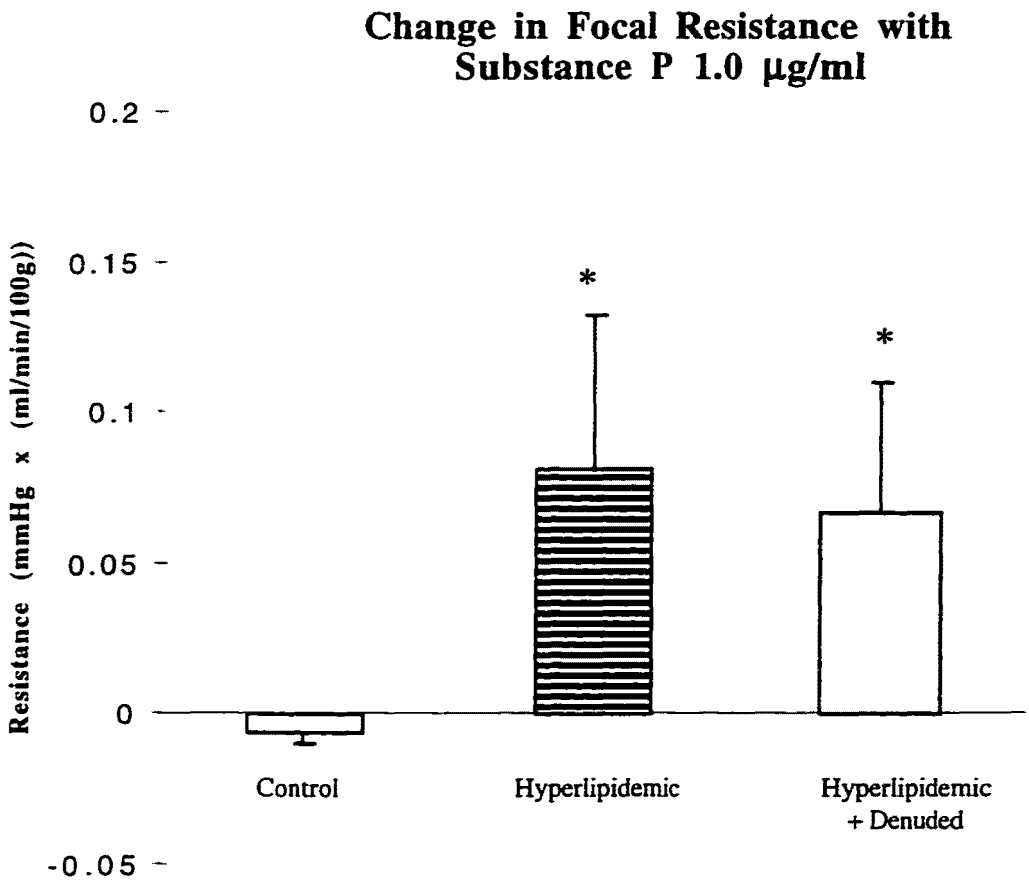


Figure 16. Change in focal resistance in response to intracoronary SP 1.0 µg/ml. (*) represents a significant difference from control, $p \leq 0.05$. The number of animals in each group is CTL (5), HC (5), and HC+D (5).

Table 14

Focal Resistance Change (mmHg·[ml/min/100g]$^{-1}$) in Response to Intracoronary Injections of Nitroglycerin (μg/ml).

Nitroglycerin 1.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	0.036±0.019	0.014±0.004	-0.036±0.033
Hyperlipidemic	0.054±0.014	0.058±0.018	0.003±0.006
Hyperlipidemic + Denuded	0.027±0.007	0.082±0.060	0.054±0.059

Nitroglycerin 2.5 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	0.063±0.027	0.020±0.005	-0.042±0.026
Hyperlipidemic	0.067±0.019	0.053±0.016	-0.014±0.005
Hyperlipidemic + Denuded	0.063±0.026	0.030±0.009	-0.032±0.030

Nitroglycerin 5.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	0.171±0.14	0.014±0.006	-0.16±0.13
Hyperlipidemic	0.067±0.019	0.039±0.015	-0.029±0.015
Hyperlipidemic + Denuded	0.038±0.017	0.027±0.013	-0.005±0.007

Nitroglycerin 10.0 μg/ml

Protocol	Baseline	Effect of Drug	Change
Control	0.021±0.0081	0.012±0.0034	-0.0088±0.0056
Hyperlipidemic	0.074±0.022	0.054±0.021	-0.022±0.018
Hyperlipidemic + Denuded	0.031±0.014	0.094±0.058	0.034±0.073

All data are represented as means±SEM (n=5/group)

DISCUSSION

A variety of research protocols have been developed to investigate the origins and pathology of atherosclerosis, many of which used hypercholesterolemia as a tool. Over the last thirty years, the low density lipoprotein particle (LDL) has been directly linked to atherogenesis. What has only recently been found concerning hyperlipidemia is that the negative effects of the condition on the vascular endothelium are evident within a relatively short period of time. Atherogenesis, progressing to the point of coronary stenosis, takes many years to develop, but recent studies suggest that myocardial ischemia can result from a defect in normal vasodilatory function.

The role of the luminal endothelium in maintaining normal vascular tone has been well established. Furchgott and Zawadzki found that removal of the endothelium in their "sandwich" preparations of arterial segments caused there to be a loss of normal vasodilation in the arterial segment [20]. Later, it was found that nitric oxide production was inhibited when the vascular endothelium was disrupted [46]. Hypercholesterolemia, whether acute or chronic, has been shown to alter normal endothelial function [4, 77, 79].

In the present study, the increases in mean and diastolic pressures with elevated levels of total cholesterol and LDL may be related to changes in endothelial function caused by elevated serum lipids in the hypercholesterolemic swine. Since normal endothelial function in brachial arteries of humans is altered after one high fat meal (Plotnick *et al*, JAMA 278:1682-86, 1997), the possibility of systemic endothelial dysfunction after two weeks of persistent significant hyperlipidemia must be considered. These results were not expected but are

consistent with the hypothesis proposed by this report. The fact that the HC+D group did not fall into the linear correlation between total cholesterol/MABP or [LDL]/diastolic pressure is not unprecedented. Studies using other agents which scavenge nitric oxide also show a saturation point at which no further effects on MABP are seen as the concentration of the drug is elevated [80]. It is possible that the serum lipid levels in the HC group were either at or above those concentrations necessary for the lipids to alter normal endothelial tone. No documentation was found that corroborated or refuted the correlation between increasing serum lipid concentration and increasing blood pressure. The absence of differences in dP/dT between groups indicates that there was no difference in ventricular work between groups, which might have occurred due to the presence of higher serum lipids and the heart's ability to utilize free fatty acids for metabolic fuel. The increase in diastolic pressure over CTL in the HC+D and in the HC group also might lend credence to the increased arterial tone being due to an increase in total peripheral resistance rather than an increase in ventricular work or cardiac output. An increase in diastolic pressure, which both HC and HC+D demonstrated over CTL, is indicative of an increase in total peripheral resistance, consistent with the hypothesis that hyperlipidemia might alter normal endothelial function.

Often the presence of hypertension in hyperlipidemic patients is attributed by clinicians to other factors more commonly associated with hypertension such as high sodium consumption or overt obesity. The present data may support another model in which hypertension in hypercholesterolemic patients is triggered by the direct effects of serum lipids on the endothelium.

The development of significantly different hyperemic responses to the pressor effects of high concentrations of acetylcholine represents a difference between the sensitivity of the CTL and HC groups to the pressor effects of ACH. Berne developed the hypothesis that the accumulation of various metabolites found in ischemic vascular beds allowed for increases in blood flow when normal flow was allowed to return (hyperemic response to vascular occlusion) [55]. Our laboratory has also demonstrated that as the myocardium increases its work, rising concentrations of adenosine are responsible for the increases in coronary blood flow [53]. The findings in the present study suggest that the condition of hyperlipidemia causes an increased sensitivity to the pressor effects of ACH as manifested in the increased hyperemic response when ACH was washed out of the coronary bed. These results suggest that high concentrations of LDL, even over only a two week period, cause the coronary vessels to become more responsive to the constrictive effects of ACH, indicating an altered endothelium in the presence of high serum cholesterol.

The significance of the ACH receptor in the coronary circulation is its apparent involvement in normal parasympathetic regulation of the coronary vasculature. Studies by this lab as well as others have show that endurance exercise increases the sensitivity of the coronary arteries to the vasoconstrictive effects of ACH [81, 82]. Post-exercise coronary arterial spasm has been linked to the re-establishment of normal parasympathetic dominance of the myocardium after an abrupt cessation of exercise [83]. Increases in serum lipids could conceivably predispose an individual to coronary arterial spasm following exercise if the sensitivity of the endothelium is already increased when exercise is abruptly stopped.

The effects of hyperlipidemia on the vascular endothelium in many clinical studies is examined by measuring the changes in either luminal diameter or by measuring the changes in flow in the large epicardial arteries. The present study not only allowed us to investigate the changes in large epicardial arteries in hyperlipidemia, but we also were permitted to quantitate changes in global and focal resistance in response to various pharmacologic challenges to hyperlipidemic swine. Thus we were able to examine changes in the arterioles of the coronary bed, the site of the greatest amount of regulation to the resistance to blood flow. The effects of hyperlipidemia on global resistance were essentially the same between HC and CTL except for the lowest concentration of ACH. The changes in global resistance with low dose ACH sheds new light on which vessels are affected by high serum cholesterol. This is consistent with the findings that in the HC and HC+D groups, an apparent increase in total peripheral resistance was noted. If this is the case, systemic arterioles, as well as those of the coronary vascular bed, might be affected by hyperlipidemia. This apparent dysfunction of the endothelium seen in an increase in sensitivity to the lowest concentration of ACH given lends more credence to the hypothesis that hyperlipidemic patients might be at greater risk for coronary arterial spasm post-exercise than a normolipic individual.

In the analysis of the changes in focal resistance to the various drugs administered, a disturbed response of the endothelium to both ACH and SP was noted, further demonstrating the dysfunction of the endothelium during conditions of hyperlipidemia. Both ACH and SP require the presence of an intact endothelium to induce a vasodilation. The process of coronary artery denudation provided us a focal region in the LAD with documented mechanically-induced

endothelial damage. This procedure is not novel to this or other laboratories when investigating atherogenesis in the swine model [84, 85]. By creating a region of known endothelial damage, we were able to compare the effects of hypercholesterolemia of the hyperlipidemic group (HC) to the CTL group and the HC+D group and identify which group the HC group resembled in focal resistance changes. In response to the two low doses of ACH, the HC group demonstrated increases in focal resistance as did the HC+D group, while the control group demonstrated a slight decrease in focal resistance. In the HC+D group, the loss of an intact endothelium predisposed the VSM to interact with the ACH and thus vasoconstrict. In the HC group, only if the endothelium was altered from normal would the vessel constrict under the same circumstances when the CTL group slightly dilated. In response to the lowest concentration of SP, we also have shown an increase in resistance from CTL in the HC and HC+D groups, with the HC and HC+D groups closely resembling one another again. The apparent increase in resistance in the HC group might be related to the ability of SP to cause VSM constriction in other parts of the body, but this effect is not usually found in arterial VSM. In rats, however, SP has been shown to have dose related effects, allowing for a decrease in peripheral resistance at low doses, but causing an increase in MABP as the concentration is increased [86]. Consistent with the finding of Angus *et al*, coronary denudation, and apparently hyperlipidemia, was shown here to disrupt the normal vasodilatory response of SP while not inhibiting vasodilation in response to organic nitrates [69].

In this study we developed a model of hypercholesterolemia that significantly increased the concentrations of the LDL particle in the serum over 14 days. In concordance with published literature, our study also demonstrated

changes in normal endothelial function in response to drugs which challenge the artery to vasodilate through endothelium-mediated means while not changing the vasodilatory effects of the endothelium-independent drugs. What we have demonstrated in this study is that in response to short term hyperlipidemia, changes in systemic vascular resistance, as well as global and focal coronary resistance to blood flow are related to the changes in normal endothelial function. These changes are demonstrated by challenging the coronary vessels to dilate in response to ACH, which has both dilatory as well as constrictor abilities. The altered response to SP also indicates a change in normal endothelial function in the hyperlipidemic swine. This study gives a new understanding to how the condition of hyperlipidemia may hinder the regulation of coronary blood flow and how it might alter normal blood flow regulation via changing normal endothelial function.

BIBLIOGRAPHY

1. Cooper, E. S. Prevention: the key to progress. Circulation 24, 1993.

2. WHO-MONICA Project. Myocardial infarction and coronary deaths in the World Health Organization: registration procedures, event rates, and care-fatality rates in 38 populations from 21 countries from four continents. Circulation 90: 583-612, 1994.

3. Heart and Stroke Facts: statistical supplement. . Dallas, TX: American Heart Association National Center, 1996.

4. Ross, R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 362: 801-809, 1993.

5. Alpert, J. S. The pathophysiology of acute myocardial infarction. Cardiology 76: 85-95, 1989.

6. Sommer, J. R., and E. A. Johnson. Ultrastructure of cardiac muscle. In: Handbook of Physiology Section 2: The Cardiovascular System, edited by R. M. Berne and N. Sperelakis. Bethesda, MD: American Physiological Society, 1979, p. 113-186.

7. Wollenberg, A. Responses of the heart mitochondria to chronic cardiac overload and physical exercise. In: Recent Studies on Cardiac Structure and Metabolism, edited by E. Bajusz and E. Rona. Baltimore: University Press, 1972, p. 213-222.

8. Braasch, W., S. Gudbjarnason, and P. S. Puri. Early changes in energy metabolism in the myocardium following acute coronary artery occlusion in anesthetized dogs. Circulation Research 23: 429-438, 1986.

9. Gudbjarnason, S., P. Mathes, and K. G. Ravens. Functional compartmentalization of ATP and creatine phosphate in heart muscle. Journal of Molecular and Cellular Cardiology 1: 325-329, 1970.

10. Wollenberger, A., and E. G. Krause. Metabolic control characteristics of the acutely ischemic myocardium. American Journal of Cardiology 22: 349-359, 1968.

11. Surrys, P. W., W. Wijins, and M. V. d. Brond. Left ventricular performance, regional blood flow, wall motion, and lactate metabolism during transluminal angioplasty. Circulation 70: 25-36, 1984.

12. Mueller, J. E. Coronary artery thrombosis. Historical aspects. Journal of the American College of Cardiology 3: 893-896, 1983.

13. Fuster, V. Lewis A. Conner Memorial Lecture: mechanisms leading to myocardial infarction: insights from studies of vascular biology. Circulation 90: 2126-2146, 1994.

14. Kawai, C. Pathogenesis of acute myocardial infarction: novel regulatory systems of bioactive substances in the vessel wall. Circulation 90: 1033-1043, 1994.

15. Ambrose, J. A., S. L. Winters, R. R. Arora, A. Eng, A. Riccio, R. Gorlin, and V. Fuster. Angiographic evolution of coronary artery morphology in unstable angina. Journal of the American College of Cardiology 78: 472-478, 1986.

16. Ambrose, J., M. Tannenbaum, D. Alexopolus, C. E. Hjemdahl-Monsen, J. Leavy, M. Weiss, S. Borrico, R. Gorlin, and V. Fuster. Angiographic progression of coronary artery disease and the development of myocardial infarction. Journal of the American College of Cardiology 12: 56-62, 1988.

17. Giroud, D., J. M. Li, P. Urban, B. Meier, and W. Rutishauer. Relationship of the site of myocardial infarction to the most severe coronary arterial stenosis at prior angiography. American Journal of Cardiology 69: 729-732, 1992.

18. Falk, E., P. K. Shah, and V. Fuster. Coronary plaque disruption. Circulation 92: 657-671, 1995.

19. Moncada, S., and A. Higgs. The l-arginine nitric oxide pathway. The New England Journal of Medicine 329: 2002-2012, 1993.

20. Furchgott, R. F., and J. V. Zawadzki. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288: 373-376, 1980.

21. Ross, R., and J. A. Glomset. Atherosclerosis and the arterial smooth muscle cell. Science 180: 1332-1339, 1973.

22. Ross, R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 362: 801-809, 1993.

23. Vesselinovitch, D., R. W. Wissler, K. Fisher-Dzoga, R. Hughes, and L. Dubien. Regression of atherosclerosis in rabbits: Part 1. Treatment with low fat diet, hyperoxia, and hypolipidemic agents. Atherosclerosis 19: 259-275, 1974.

24. Zilversmit, D. B. Atherogenesis: a postprandial phenomenon. Circulation 60: 473-485, 1979.

25. Goldstein, J. L., and M. S. Brown. The low density lipoprotein pathway and its role in atherosclerosis. Annual Review of Biochemistry 46: 897-930, 1977.

26. Esterbauer, H., J. Gabicki, H. Puhl, and G. Jürgens. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. Free Radical Biology and Medicine 13: 341-390, 1992.

27. Myant, N. B. Cholesterol Metabolism, LDL and the LDL Receptor. . San Diego: Academic Press, 1990, p. 99-111.

28. Goldstein, J. L., and M. S. Brown. Progress in understanding the LDL receptor and HMG-CoA reductase, two membrane proteins that regulate the plasma cholesterol. Journal of Lipid Research 25: 1450-1461, 1984.

29. Berg, K., L. M. Powell, S. C. Wallis, R. Pease, T. J. Knott, and J. Scott. Genetic Linkage between the antigenic group (Ag) variation and the apolipoprotein B gene: assignment of the Ag locus. Proceedings of the National Academy of Science, USA 83: 7367-7370, 1986.

30. Brown, M. S., and J. L. Goldstein. A receptor-mediated pathway for cholesterol homeostasis. Science 232: 34-47, 1986.

31. Frank, J. S., and A. M. Fogelman. Ultrastructure of the intima in WHHL and cholesterol fed rabbit aortas prepared by ultra-rapid freezing and freeze etching. Journal of Lipid Research 30: 967-978, 1989.

32. Kruth, H. S. Subendothelial accumulation of unesterified cholesterol: an early event in the atherosclerotic lesion development. Atherosclerosis 57: 337-341, 1985.

33. Smith, E. B. The relationship between plasma and tissue lipids in human atherosclerosis. Advances in Lipid Research 12: 1-49, 1974.

34. Hoff, H. F., and C. L. Heideman. Apolipoprotein B retention in the grossly normal and atherosclerotic human aorta. Circulation Research 41: 684-690, 1977.

35. Mehrabian, M., L. L. Demer, and A. J. Lusis. Differential accumulation of intimal monocyte/macrophages relative to lipoproteins and lipofuscin corresponds to hemodynamic forces on cardiac valves in mice. Arteriosclerosis and Thrombosis 11: 947-957, 1991.

36. Demer, L. L., C. M. Wortham, E. R. Dirksen, and M. J. Sanderson. Mechanical stimulation induces intracellular calcium signaling in bovine aortic endothelial cells. American Journal of Physiology 264: H2094-H2102, 1993.

37. Cornhill, J. F., E. E. Herderick, and A. M. Gotto. Topography of human sudanophillic lesions. Monographs of Atherosclerosis 15: 13-29, 1990.

38. Gerrity, R. G., H. K. Naito, M. Richardson, and C. J. Schwartz. Dietary induced atherogenesis in swine. American Journal of Pathology 95: 775-793, 1979.

39. Masuda, J., and R. Ross. Atherogenesis during low level hypercholesterolemia in the non-human primate, I: fatty streak formation. Arteriosclerosis 10: 164-177, 1990.

40. Harland, H. A., J. D. Gilbert, and C. J. Brooks. Lipids of human atheroma. Biochim. Biophys. Acta 316: 378-375, 1973.

41. Fogelman, A. M., I. Shechter, J. Seager, M. Hokom, J. S. Child, and P. A. Edwards. Malondialdehyde alteration of low density lipoproteins leads to cholesterol accumulation in human monocyte/macrophages. Proceedings of the National Academy of Sciences, USA 77: 2214-2218, 1980.

42. Cookson, F. B. The origin of foam cells in atherosclerosis. British Journal of Experimental Pathology 52: 62, 1971.

43. Salonen, J. T., N. Kristiina, R. Salonen, E. Porkkala-Sarataho, T. P. Tuomainen, U. Diczfalusy, and I. Bjökhem. Lipoprotein oxidation and progression of carotid atherosclerosis. Circulation 95: 840-845, 1997.

44. Nilsson, J. Growth factors and the pathogenesis of atherosclerosis. Atherosclerosis 62: 185-189, 1986.

45. Faggiotto, A., R. Ross, and L. Harker. Studies of hypercholesterolemia in the nonhuman primate. I. Changes that lead to fatty streak formation. Arteriosclerosis 4: 323-340, 1984.

46. Palmer, R. M. J., A. G. Ferrige, and S. Moncada. Nitric oxide release accounts for the biologic activity of endothelium-derived relaxing factor. Nature 327: 524-526, 1987.

47. Lloyde-Jones, D. M., and K. D. Bloch. The vascular biology of nitric oxide and its role in atherogenesis. Annual Review of Medicine 47: 365-375, 1996.

48. Lincoln, T. M. Cyclic GMP and mechanisms of vasodilation. Pharmacological Therapeutics 41: 479-502, 1989.

49. Gardiner, S. M., A. M. Compton, and T. T. Bennett. control of regional blood flow by endothelium-derived nitric oxide. Hypertension 15: 486-492, 1990.

50. Ardehali, A., and T. A. Ports. Myocardial oxygen supply and demand. Chest 98: 699-705, 1990.

51. McKenzie, J. E., R. P. Steffen, and F. J. Haddy. Regulation of coronary blood flow during increased cardiac work. In: Ca+2 Entry Blockers, Adenosine, and Neurohumors, edited by G. F. Merill and H. R. Weiss. Baltimore: Urban and Schwarzenberg, 1981, p. 237-248.

52. Headrick, J. P., and R. J. Willis. Relation between O2 supply:demand ratio, MVO2, and adenosine formation in hearts stimulated with inotropic agents. Canadian Journal of Physiology and Pharmacology 68: 110-118, 1990.

53. McKenzie, J. E., E. L. Bockman, R. P. Steffen, F. P. McCoy, and F. J. Haddy. Transmural adenosine with increased cardiac work. Basic Research in Cardiology 76: 372-376, 1981.

54. Berne, R. M. The role of adenosine in the regulation of coronary blood flow. Circulation Research 47: 807-813, 1980.

55. Berne, R. M. Cardiac nucleotides in hypoxia: possible role in regulation of coronary blood flow. American Journal of Physiology 204: 317-322, 1963.

56. Cohen, R. A., K. M. Zitnay, C. C. Haudenschild, and L. D. Cunningham. Loss of selective endothelial cell vasoactive functions caused by hypercholesterolemia in pig coronary arteries. Circulation Research 63: 903-910, 1988.

57. O'Driscoll, G., D. Green, and R. R. Taylor. Simvastatin, an HMG-coenzyme A reductase inhibitor, improves endothelial function within one month. Circulation 95: 1126-1131, 1997.

58. Anderson, T. J., I. T. Meredith, F. Charbonneau, A. C. Yeung, A. P. Selwyn, and P. Ganz. Endothelium-dependent coronary vasomotion relates to the susceptibility of LDL to oxidation in humans. Circulation 93: 1647-1650, 1995.

59. Murohara, T., K. Kugiyama, M. Ohgushi, S. Sugiyama, Y. Ohta, and H. Yasue. LPC in oxidized LDL elicits vasoconstriction and inhibits endothelium-dependent relaxation. American Journal of Physiology 36: H2441-H2449, 1994.

60. Cowan, C. L., and J. E. McKenzie. Cholinergic regulation of resting coronary blood flow in domestic swine. American Journal of Physiology 259: H109-H115, 1990.

61. Christie, M. I., T. M. Griffith, and M. J. Lewis. A comparison of basal and agonist-stimulated release of endothelium-derived relaxing factor from different arteries. British Journal of Pharmacology 98: 397-406, 1989.

62. Brum, J. M., Q. Sufan, G. Lane, and A. Bove. Increased vasoconstrictor activity of proximal coronary arteries with endothelial damage in intact dogs. Circulation 70: 1066-1073, 1984.

63. Busse, R., A. Mülsch, I. Fleming, and M. Hecker. Mechanisms of nitric oxide release from the vascular endothelium. 1993 87 [supplement V]: V18-V25, 1993.

64. Freiman, P. C., G. G. Mitchell, D. D. Heistad, M. L. Armstrong, and D. G. Harrison. Atherosclerosis impairs endothelium-dependent vascular relaxation to acetylcholine and thrombin in primates. Circulation Research 58: 783-789, 1986.

65. Crossman, D. C., S. W. Larkin, R. W. Fuller, G. J. Davies, and A. Maseri. Substance p dilates epicardial coronary arteries and increases coronary blood flow in humans. Circulation 80: 475-484, 1989.

66. Fuller, R. D., D. L. Maxwell, D. M. S. Dixon, G. P. McGregor, V. F. Barnes. S. R. Bloom, and P. F. Barnes. The effect of substance P on cardiovascular and respiratory function in normal subjects. Journal of Applied Physiology 62: 1473-1479, 1987.

67. Ezra, D., F. R. M. Laurindo, J. Eimeri, R. E. Goldstein, C. C. Peck, and G. Feuerstein. Tachykinin modulation of coronary blood flow. European Journal of Pharmacology 122: 135-138, 1986.

68. Boulanger, C., R. R. Lorenz, H. Hendrickson, and P. M. Vanhoutte. Release of different relaxing factors by cultured porcine endothelial cells. Circulation Research 64: 1070-1078, 1989.

69. Angus, J. A., A. Mitani, Y. Kitajima, T. Ohno, and T. Ishihara. Difference in pressor responses to NG-monomethyl-L-arginine between conscious and anesthetized rats. Japanese Journal of Pharmacology 56: 245-248, 1991.

70. Steinbrecher, U. P., S. Parthasarathy, D. S. Leake, J. L. Witztum, and D. Steinberg. Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipids. Proceedings of the National Academy of Sciences, USA 81: 3883-3887, 1984.

71. Andrews, H. E., K. R. Bruckdorfer, R. C. Dunn, and M. Jacobs. Low-density lipoproteins inhibit endothelium-dependent relaxation in rabbit aorta. Nature 327: 327-329, 1987.

72. Treasure, C. B., J. L. Klein, W. S. Weintraub, J. D. Talley, M. E. Stillabower, A. S. Kosinski, J. Zhang, S. J. Boccuzzi, J. C. Cedarholm, and R. W. Alexander. Beneficial effects of cholesterol-lowering therapy on the coronary endothelium in patients with coronary artery disease. The New England Journal of Medicine 332: 481-487, 1995.

73. Group, S. S. S. S. Randomized trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). Lancet 344: 1383-1389, 1994.

74. Meredith, I. T., A. C. Yeung, F. F. Weidinger, T. J. Anderson, A. Uehata, T. J. Ryan, A. P. Selwyn, and P. Ganz. Role of endothelium-dependent vasodilation in ischemic manifestations of coronary artery disease. Circulation 87[supplement V]: V56-V66, 1993.

75. Bolibar, I., S. G. Thompson, A. v. Eckardstein, M. Sandkamp, and G. Assmann. Dose-response relationships of serum lipid measurement with the extent of coronary stenosis. Arteriosclerosis, Thrombosis, and Vascular Biology 15: 1035-1042, 1995.

76. Berliner, J. A., N. Mohamad, A. M. Fogelman, J. S. Frank, L. L. Demer, P. A. Edwards, A. D. Watson, and A. J. Lusis. Atherosclerosis: basic mechanisms. Oxidation, Inflammation, and genetics. Circulation 91: 2488-2496, 1995.

77. Potnick, G. D., M. C. Corretti, and R. A. Vogel. Effects of antioxidant vitamins in the transient impairment of endothelium-dependent brachial artery vasoactivity following a single high fat meal. Journal of the American Medical Association 278: 1682-1686, 1997.

78. Motoyama, T., H. Kawano, K. Kugiyama, O. Hirashima, M. Ohgushi, M. Yoshimura, H. Ogawa, and H. Yasue. Endothelium-dependent vasodilation in the brachial artery is impaired in smokers: effect of vitamin C. American Journal of Physiology 273 (4Pt2): pH1644-H1650, 1997.

79. Lekakis, J., C. Papamichael, C. Vemmos, J. Nanas, D. Kontoyannis, S. Stamatelopoulos, and S. Moulopoulos. Effect of acute cigarette smoking on endothelium-dependent brachial artery dilation in healthy individuals. American Journal of Cardiology 79: 529-531, 1997.

80. Barve, A., A. P. Sen, P. R. Saxena, and A. Gulati. Dose response effects of diasprin crosslinked hemoglobin (DCLHb) on systemic hemodynamics and regional blood circulation in rats. Artificial Cells, Blood Substitutes, and Immobilization Biotechnology 25: 75-84, 1997.

81. Tondi, B. The effects of endurance exercise training on the coronary vascular responsiveness to intracoronary acetylcholine in swine. In: Physiology. Bethesda: Uniformed Services University of the Health Sciences, 1993, p. 112.

82. Friedman, M. H. P., and S. R. A. Friedman. Influence of exercise "warm-up"/"cool down" on heart rate. FASEB J. 4: 5411, 1990.

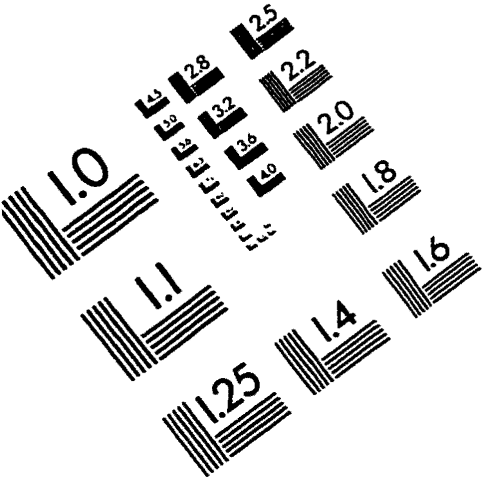
83. Sandaniantz, A., D. Kitzes, and P. D. Thompson. Post-exercise coronary artery spasm. American Heart Journal 116: 866-867, 1988.

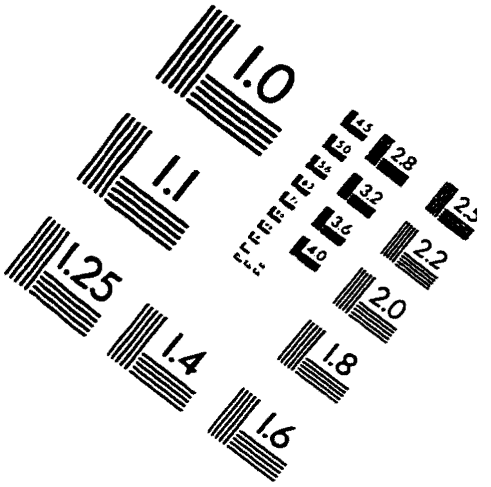
84. Ahle, N. Endothelial mediation of coronary vascular tone: Nitric oxide attenuation of cholinergic vasospastic challenge. In: Physiology. Bethesda: Uniformed Services University of the Health Sciences, 1993, p. 78.

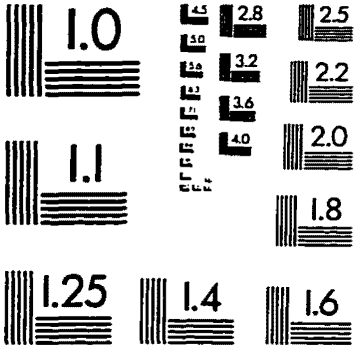
85. Nunes, G. L., D. S. Sgoutas, R. A. Redden, S. R. Sigman, M. B. Gravanis, S. B. King, and B. C. Berk. Combination of Vitamins C and E Alters the Response to Coronary Balloon Injury in the Pig. Arterioscler Thromb Biol 15: 156-165, 1995.

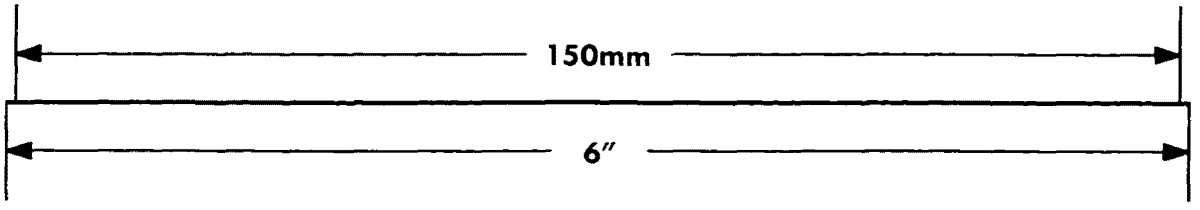
86. Hancock, J. C. Hemodynamic actions of substance P in anesthetized rats. In: Substance P and Neurokinins, edited by J. L. Henry, R. Couture, A. C. Cuello, G. Pelletier, R. Quirion and D. Regoli. New York: Springer-Verlag, 1987, p. 164-165.

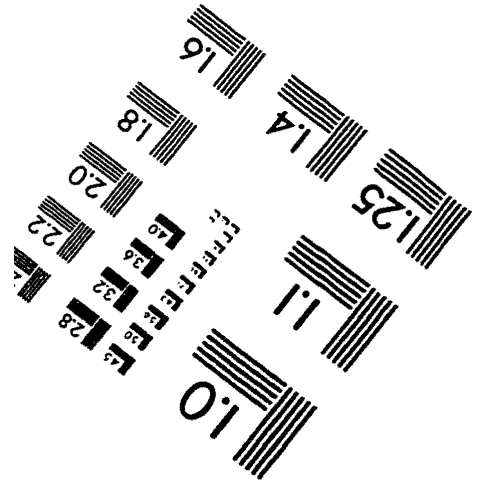
IMAGE EVALUATION TEST TARGET (QA-3)

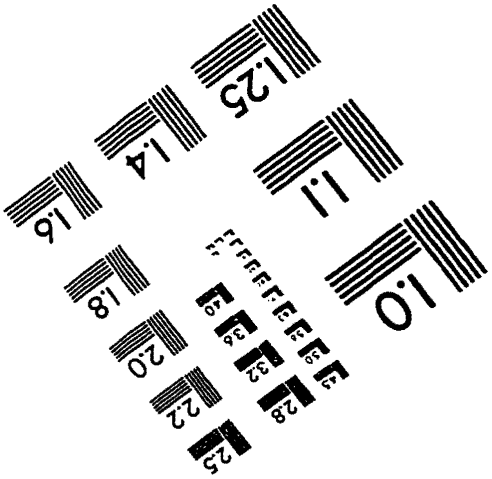












APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989

© 1993, Applied Image, Inc., All Rights Reserved